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STUDIES ON THE SYMBIOSIS OF THE BODY LOUSE

I. ELIMINATION OF THE SYMBIONTS BY CENTRIFUGALISATION OF THE EGGS

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(With Plate XII)

IN a very great number of arthropods there are regularly found certain micro-organisms which, owing to their intracellular life, closely resemble parasites. The invasion differs, however, from a true parasitic one in that the micro-organisms are regularly transmitted to the offspring of the host and are present in fairly constant numbers in all members of the same species.

Buchner and his co-workers, who have made the most extensive and detailed study of this subject, concluded from the morphological analysis of the life cycles of a very great number of these micro-organisms that this type of relationship cannot be classified as either parasitism or commensalism. The regularity of appearance and the intimacy of the mutual adaptation find, according to Buchner, a satisfactory explanation only if we suppose that there exists a relationship between the arthropod and the micro-organisms which is mutually beneficial, or in other words is a true symbiosis. According to this view it is essential that both parties to the relation must benefit. On the one hand the micro-organisms by their presence provide some necessary requirement of the arthropod while they, on the other hand, are themselves enabled to multiply. Facilities for the multiplication of the micro-organisms and for their transmission to the progeny of the arthropod are provided by the development of a special organ called the mycetome.

The fundamental difference between this relationship and that of parasitism lies in the lack of mutual benefit in the latter case where one party lives entirely at the expense of the other.

Buchner's view has not been generally accepted. Doubts have been expressed as to whether a symbiotic relationship can be definitely postulated on the basis of morphological findings alone, and the suggestion has been made that some other explanation exists.

It is still believed, especially among parasitologists, that all relations between host and inhabiting micro-organisms can be expressed as parasitism. According to this view, the so-called symbionts are more or less harmless commensals, while the bacterial organs, the mycetomes, are considered as formations analogous to the plant gall produced by the host as a response to the irritation caused by the foreign inhabitant.

The problem to be solved is whether the association of the two unequal partners is a mere accident, or whether it serves a useful purpose. This can only be decided by experiment. If an experimental separation of the micro-organism from its host causes injury to the latter, and if it can be shown that the only cause of this injury is the absence of the micro-organism, then the existence of a true symbiosis between the host and its micro-organisms, as claimed by Buchner, would be definitely proven.

The object of this paper is to describe a simple method of eliminating the symbionts from the louse, a typical symbiont host, and the effect of this elimination on the host.

The symbiosis in the louse has been observed independently by Sikora (1919) and Buchner (1920). The life cycle of the symbiont has been worked out and described in detail by Ries (1932). From his description, which in the course of our work we were able to confirm in all essential particulars, several facts may be cited which are necessary in order to understand the principle of our method.

In the larva of the body louse, all symbionts are concentrated in a small organ, which is called *Magenscheibe* (stomach disc) by the German authors. The organ is round or oval in shape and is fixed to the outer ventral mid-gut, where it always lies exactly in the mid-ventral line of the body, slightly nearer the anal region than the head (Fig. 1). This organ is composed of cells derived from the mid-gut, it is covered with an irregular outer layer of mesodermal tissue, and encloses a more or less spherical cavity. This cavity is formed during the embryonic development of the louse as part of the stomach lumen, but after the larva is hatched it has no open connection with the lumen of the gut. It is divided radially into 12–14 chambers enclosing the symbionts (Fig. 7).

The whole organ has an opaque, yellowish colour, produced by certain granules of high refraction index, enclosed in the inner cells. Because of its colour, which stands out sharply against the dark red background of the blood-filled stomach, it can easily be recognised in the living animal even with an ordinary hand lens.

Prior to the formation of the stomach disc in the embryo the symbionts are enclosed in a group of cells which float freely in the yolk inside the lumen of the mid-gut. This group of cells, together with the enclosed symbionts, is called the primary mycetome. The symbionts are transmitted from the primary mycetome inside the mid-gut to the secondary mycetome, the stomach disc, in the following manner. The primary mycetome migrates to the place on the ventral mid-gut wall where later the stomach disc is formed. Here the wall of the mid-gut is pushed out in a hernia-like fashion so that a pocket formation results which includes the primary mycetome with its symbionts. This pocket is closed later in such a way that the symbionts are retained in the newly formed cavity and the cells of the primary mycetome are pushed back into the yolk where they degenerate and are finally dissolved. The newly formed cavity containing the symbionts is then divided by radial septa into chambers and thus the previously described stomach disc results. In the male host the symbionts remain in the stomach disc from the larva stage until its death, while in the female host a few days before the imago stage all the symbionts emigrate from the stomach disc and infect a certain region in the oviducts. This region later develops into the so-called ovarian ampules.

In the present work the effect of centrifugalisation upon the developing eggs of lice was studied. It was found that eggs which had been exposed to centrifugalisation between the second and the fifth day of development (1500–2000 revolutions per minute, radius 20 cm., for about 8 hours daily) yielded

5–10 per cent. of larvae with displaced stomach discs[1]. In some of them the mycetome was on the dorsal side of the stomach instead of in the usual position on the ventral mid-gut. In others this organ was definitely displaced towards the anal region or towards the lateral parts of the body (Figs. 2–6).

Sometimes the mycetome was actually divided into two parts. In such a case one part was always situated in the normal place, while the position of the other was very variable; in some, both parts were on the ventral side, in others one was on the ventral, the other on the dorsal side. More than two parts were never observed.

No other change than the displacement of the mycetome could be detected outwardly; histological examination, however, showed that all the displaced mycetomes, and only those, were entirely free from symbionts. The structure of the displaced mycetomes differed from normal in that the regular division into chambers was replaced by an irregular, more or less compact, massing of cells. The mycetome itself, as in normal organs, consisted of cells derived from the mid-gut, which were covered by an irregular layer of mesodermal elements.

Occasionally irregular spaces were observed inside these organs, filled with a granular mass, which consisted, most probably, of the degenerated symbionts; but intact micro-organisms were never found in these animals, either in the mycetomes or elsewhere. In other words the larvae with the displaced mycetomes were absolutely free of symbionts while the other larvae in which the stomach disc was not displaced had their normal symbiont population.

In former papers (Aschner, 1932; Aschner and Ries, 1933) we were able to show that extirpation of the stomach disc at the time when it was filled with symbionts caused grave deficiency symptoms, while the same operation performed after migration of the symbionts to the oviduct had no influence at all on the well-being of the louse. From these results it was concluded that it was not the operation, nor the lack of the mycetome, but rather the absence of symbionts that caused the observed deficiency symptoms.

The larvae which had been freed from their symbionts by centrifugalisation of the egg offered an opportunity to test the above conclusion. In the previous experiments we studied lice lacking both mycetome and symbionts, and lice without mycetome but with symbionts; in the present experiment it was possible to follow the fate of lice in which the mycetome was present and the symbionts absent.

The centrifugalised larvae, normal, as well as those free of symbionts, were kept together constantly in the same breeding cage, under absolutely identical conditions. At the beginning no difference was observed among the two groups. All larvae fed and developed normally until the fifth or sixth day. Then the symbiont-free larvae died suddenly. At this time first-stage larvae usually

[1] As no laboratory centrifuge which could be used for so long a time without interruption was available, the eggs were fixed in various positions to the inner side of the transmission wheel of a dynamo which worked that number of hours daily. The eggs did not suffer from this treatment and a large percentage hatched even when fixed to the wheel for the whole period of development.

moult into second-stage larvae, but there does not seem to be any connection between the moulting and the sudden death of the symbiont-free larvae. It is true that most of them died immediately before or during the moulting, but some of them succeeded in passing through this process, only to exhibit the same symptoms shortly afterwards. It would seem, therefore, that the death of the symbiont-free lice is related to a time interval rather than to the process of moulting.

These observations are convincing confirmation of the previous results obtained by an entirely different method. There, too, we demonstrated that after the elimination of the symbionts the *Pediculus* larvae can live normally for only about six days and die more or less suddenly after that time.

In cases where the mycetome was divided by centrifugalisation into two parts, the part situated in its normal place had its share of symbionts while the displaced part was free of them. In these larvae only a part of the symbionts was eliminated, and the condition created was the same as that obtained by partial extirpation of the mycetome. The quantity of symbionts in this case depends only upon the relative size of the two parts, the smaller the displaced part of the mycetome the smaller the number of symbionts eliminated. In cases of partially displaced mycetomes, the lice lived longer than those which were entirely sterile but never as long as normal lice. The results are, therefore, in complete accord with those obtained with larvae with a partially extirpated mycetome.

The reason for the sudden death of the symbiont-free larvae will be dealt with in another paper. Here we must deal with the problem of how the centrifugal force is able to displace the mycetome and why the symbionts disappear.

At first it was supposed that centrifugalisation forced the primary mycetome, while still floating in the yolk, inside the mid-gut, away from its normal place on the inner mid-gut wall. But histological examination of the displaced mycetomes did not support this assumption. In most cases there was no trace of a connection between the epithelium of the stomach and the corresponding cells of the mycetome; it seems, therefore, very improbable that the mycetome developed from the mid-gut epithelium at these places. Furthermore, this theory does not explain why the same centrifugal force should push one part of the mycetome to the dorsal and the other to the ventral side of the stomach. Nor does this assumption explain the fact that in cases of division of the stomach disc one part always remained in its normal place.

In order to explain these different facts it must be assumed that the centrifugal force affects the stomach disc and not the primary mycetome. In all cases the primary mycetome reached its normal destination on the inner ventral side of the mid-gut, where the stomach disc developed in the usual way. It is only after the formation of this organ that the centrifugal force is effective in totally or partially uprooting and displacing it. The final shape and location of this organ depends on the position of the egg during the centrifugalisation and the duration of the deforming effect of this process. As soon as the latter

ceases, the organ remains unchanged, is covered in the usual way with mesodermal cells and becomes fixed wherever it happens to be.

This explanation anticipates that the stomach disc in the embryo is of a quasi-plastic consistency. This probably does not hold true for the organ in the larval stage, but it is quite possible that in the process of formation its condition is different from that in the final stage. Indeed in examining a large number of centrifugalised larvae the "dripping" of the stomach disc can be seen in various intermediate stages. The typical drop-shaped mycetome, shown in Fig. 4, illustrates this process especially well. If the centrifugal force in this case had acted a little longer two rounded mycetomes would have resulted as shown in Fig. 5.

If the centrifugal force, due to the position of the egg, acted simultaneously in a lateral as well as in a dorsal direction, then the mycetome was shifted to its new position by floating around outside the wall and, in cases where only a part of the stomach disc was pushed away, larvae resulted with one mycetome on the dorsal side and another on the ventral side of the mid-gut (Fig. 2).

The peculiar fact that the centrifugal force affects the stomach disc only, while all the other organs, including the primary mycetome, are not disturbed in the slightest way, is most probably due to the granules already described which develop only in the cells of this organ. These granules, from their behaviour towards different acids, consist most probably of calcium oxalates. There is no doubt that the specific gravity of the cells harbouring these granules is greater than that of all the other tissues, and consequently these cells are more affected by the centrifugal force than the rest of the embryo.

The reason for the disappearance of the symbionts in the displaced mycetome is still unknown. We can only surmise that the uprooting of the mycetome from its original place causes certain changes and that the symbionts are unable to survive even for a short time under these changed conditions.

This clearly shows that the formation of the mycetome in the louse cannot be compared with the encapsulation of a focus of parasitic infection, as observed in higher animals. In the latter a disturbance of the existing equilibrium would as a rule cause a spreading of the parasites to the non-infected parts of the host. In the louse the mycetome seems to be rather a protecting pocket for the micro-organisms which are unable to live in any other part of the host.

That the mycetome could not be explained as a gall formation was shown by the former experiments on the extirpation of the stomach disc. We were able to demonstrate that the different mycetomes of the louse, the primary, the stomach disc, and, in the female, the ovarial ampules, were formed in the usual way without the possible irritating effect of the symbionts present (Figs. 8 and 9).

SUMMARY AND CONCLUSIONS

A simple method is described for eliminating the symbionts from the body louse during the egg stage, and thus producing symbiont-free larvae. The

behaviour of lice freed of their symbionts by this method is described and compared with that of lice freed of their symbionts by extirpation of the larval mycetome.

The complete accord of the results obtained by the different methods warrants the conclusion that the symbionts play an essential rôle in the life of the louse, so much so that without them death of the larvae results. It is, therefore, justifiable to consider this relationship true symbiosis.

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EXPLANATION OF PLATE XII

Fig. 1. Ventral view of a normal *Pediculus* larva showing the position and shape of the stomach disc.

Fig. 2. Ventral and dorsal view of a louse with a partially displaced stomach disc. Only a small portion of the mycetome remained on the ventral side (*a*), while the larger part is situated on the dorsal side (*b*).

Figs. 3–5. Different stages of a partial displacement of the stomach disc in three different lice. Ventral views.

Fig. 3. Beginning of the deformation of the stomach disc.

Fig. 4. Drop-shaped stomach disc. The displaced part is still connected with the remaining part of the mycetome.

Fig. 5. The displaced part is shown definitely separated from the remaining part.

Fig. 6. The whole stomach disc displaced toward the anal region.

Fig. 7. Sagittal section through the stomach disc of a normal louse embryo. The formation of the chambers with the symbionts clearly visible inside. In the yolk are visible the empty cells of the primary mycetome with the first signs of degeneration.

Fig. 8. Sagittal section through the stomach disc of a symbiont-free louse embryo where mother was freed of its symbionts in the third larval stage by extirpation of the stomach disc. Formation of the chambers is the same as in the normal embryo, but inside the chambers fragments of the primary mycetome and single yolk clumps are present instead of the symbionts.

Fig. 9. Section through the ovarian ampule of a *Pediculus* female freed of its symbionts in the third larval stage. In a normal female the symbionts would be found in the large group of cells where the ovarioles join together.

Fig. 10. Section through a displaced stomach disc (on the dorsal side of a larva). The irregular compact massing of cells instead of the regular chamber as in Fig. 7 is shown. There are no symbionts present.

Magnification: Figs. 1–6 ×25; Figs. 7–9 ×250; Fig. 10 ×300.

Preparations are stained with iron-haematoxylin.

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Fig. 1

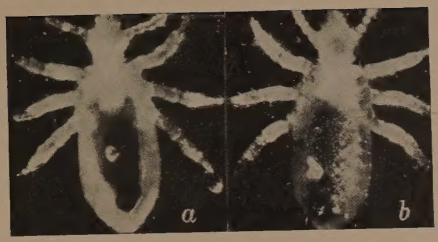

Fig. 2

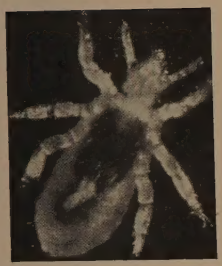

Fig. 3

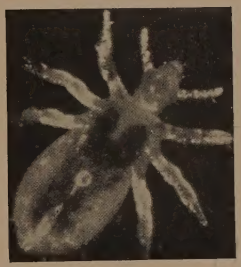

Fig. 4

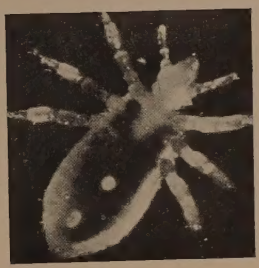

Fig. 5

Fig. 6

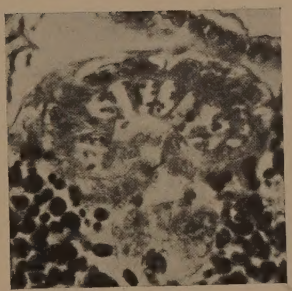

Fig. 7

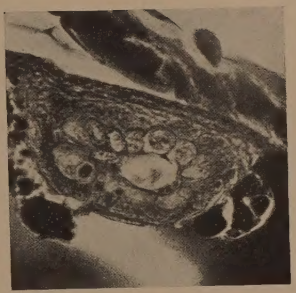

Fig. 8

Fig. 9

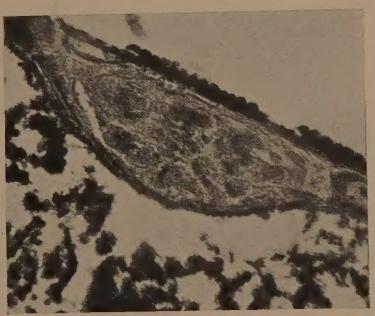

Fig. 10

ON THE EMPLOYMENT OF VOLUNTEERS IN TRYPANOSOMIASIS RESEARCH; AND ON THE ELEMENT OF CONTROL IN EXPERIMENTS WITH TRYPANOSOMES AND GLOSSINAE

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The two subjects considered in this paper are of practical importance to those interested in research of the kind carried out at the Human Trypanosomiasis Research Institute, Uganda. Their discussion would seriously encumber a report on experimental work and would distract the reader's attention from the main theme. The subjects are therefore dealt with separately, since they bear on all work, past and present, carried out at the Institute.

I. On the employment of volunteers

In any discussion on the zoological status of *Trypanosoma brucei*, *T. gambiense* and *T. rhodesiense* it is generally premised that *T. brucei* cannot infect man whereas the two latter can. About *T. gambiense* scientific opinion is at all events unanimous. It is a trypanosome whose chief mammalian host is man, and it is carried in nature by those species of tsetse that in their normal environment regularly use man as a source of food. The position of *T. rhodesiense* is more obscure. Those who hold that this trypanosome derives from *T. gambiense* and is zoologically distinct from *T. brucei* presumably believe *T. rhodesiense* to be always pathogenic to man. According to this view the power of infecting man is a deeply rooted quality of the trypanosome and possesses specific value, an assumption that receives some support from the researches carried out recently by Yorke and his collaborators at Liverpool. If, on the other hand, *T. rhodesiense* is a variety of *T. brucei* that is capable of using man as a host, it will not be surprising if in certain circumstances this distinguishing character is lost and the trypanosome reverts to a normal, "inoffensive" to man, *T. brucei*. The true affinities of all these trypanosomes can only be revealed by direct experiments on man himself, and until the last year or so the risks connected with such experiments precluded their employment on anything like an adequate scale.

Until a few months ago it had never been proved experimentally that either *T. gambiense* or *T. rhodesiense* could be transmitted cyclically to man by tsetse. There was plenty of indirect epidemiological evidence that this process did occur commonly in nature, especially in *T. gambiense* regions; but for all the experimental evidence to the contrary, the human trypanosomes might owe

their spread more to direct than to cyclical transmission. Recent advances in chemotherapy have removed the main obstacle to experiments on man, and it is now safe to use volunteers. As is customary in an enterprise of this nature, Europeans first came forward. Dr J. F. Corson in 1930, having become infected accidentally with *T. rhodesiense* by wild fly, was treated and cured without leaving his post. A year or so later he inoculated himself with the same species of trypanosome and once more, with a minimum of disturbance, demonstrated the complete efficacy of Bayer 205 in an early infection (Corson, 1932). Quite recently, Dr H. Fairbairn, in Tanganyika Territory, infected himself by injection, and again Bayer 205 brought about a complete recovery (Fairbairn, 1933).

The employment of native African *volunteers* began with certain investigations carried out at Entebbe two years ago, and in the account of that work a full description of the enrolment of these men was given (Duke, 1932).

At the stage to which our knowledge has now advanced all will, I think, admit that it is in practice impossible to settle the unsolved problems of human trypanosomiasis without the assistance of native Africans: the number required alone justifies this assertion. Once he has been infected and cured, a volunteer cannot participate again for a considerable time because of the preventive effect exerted by the Bayer 205 used in treatment—a matter now under investigation at Entebbe. Up to the present, in the investigations in progress in Uganda into the antelope reservoir and the prophylactic effects of Bayer 205, twenty-four native volunteers have been employed. Before their engagement all these men fully understood the significance of their contract. So far, sixteen of them have become infected and there has been no untoward event in the subsequent career of any of them. All would-be volunteers are carefully examined before enrolment and several candidates have been rejected for cardiac and other defects. No sort of secrecy, deception or compulsion has been used throughout the manipulations; everyone knew the moment that a colleague was taken off to hospital, and everyone was free to visit him in the wards. The only complaint—if it can be so styled—ever advanced by any of these natives was regret, on the part of those who escaped infection, at their failure to earn the reward attaching to the consummated sacrifice.

In the work on the antelope reservoir and on the prophylactic effect of Bayer 205, the regular method of infection employed is the bite of a cyclically infected fly, or, in rare instances where this is impossible, the subcutaneous inoculation of its glands. In all previous experiments on this subject inoculation of infected blood by the syringe has been used, a method whose only approximate equivalent in nature is direct transmission. There is some evidence to show that failure to infect by direct inoculation of infected blood is not strictly equivalent to failure to infect with metacyclic forms by fly-bite. More significant still, there is evidence, as yet unpublished, that the mammal from which the trypanosome is taken may influence the ability of the parasite to infect man, at all events when cyclically infected tsetse are employed. Until all these

points are clear the safest course is, therefore, to follow as closely as possible the natural mode of infection by using cyclically infected tsetse, reserving direct inoculation for special cases where, for example, a strain under investigation is non-transmissible by *Glossina*. There are, too, other possible objections, of an ethical nature, against the inoculation of blood.

Several observers in different parts of the world have deliberately inoculated themselves with trypanosome-containing blood and others have become infected accidentally in European laboratories. But all these experiments were performed with trypanosomes of animals as distinct from those of known human origin. No one, before Corson, had ever tested on man a trypanosome known to have been at one time pathogenic to man.

In addition to the more obvious points to be considered in connection with the use of volunteers, there is a theory recently advanced by Corson (1934). He suggests that there may be a state of cryptic trypanosomiasis set up in man by strains of *T. rhodesiense* that have been exposed for long periods to the tissues of resistant animals such as antelope. This possibility was suggested to him by an experiment wherein a white rat was inoculated on October 13th, 1932, with a strain of *T. brucei*. Examination of fresh films of this rat on October 17th, 22nd, 25th, 27th, 31st, November 12th and December 9th, 1932, and March 21st, 1933, failed to reveal trypanosomes; and similarly with thick blood films examined on October 19th and December 9th, 1932. But on June 24th, 1933, the day of the animal's death, the blood was found to be swarming with trypanosomes. The strain used is described as possessing "unusually low virulence for white rats." In the table of animal experiments, in several rats the incubation period was prolonged—from 12 to 22 days, and in one animal from 1 to 2 months—and several of the inoculated rats never became infected. Lavier's experience with white rats infected with *T. gambiense* shows that mild strains of trypanosomes in these animals can at times only be demonstrated by exhaustive examination of the blood, films being taken daily, fixed and stained (Lavier, 1928). It therefore seems possible that the explanation of the behaviour of this rat is that the first appearance of trypanosomes escaped notice.

On several occasions during the investigations in which the volunteers were employed, when the bite of a known infective fly failed to infect a man, an inoculation of 10 c.c. of his blood was made into a clean monkey on or about the 21st day after exposure to the fly. All such tests were negative. Medical observers in the field in Tanganyika Territory, whose experience of *T. rhodesiense* is unrivalled, have, I understand, never yet met with an example of this hypothetical cryptic infection. Moreover, the behaviour of all the volunteers who have become infected at Entebbe goes to show that when man does become infected with *T. rhodesiense*, whatever be the immediate antecedents of the trypanosome, there is no mistake about it. In every case definite symptoms were observed; high fever, malaise, and in several instances local lesions at the site of the bite.

After a careful examination of Dr Corson's paper, the evidence seems to me insufficient to warrant postponement of experiments with man, especially as the very nature of these investigations demands that wherever possible every volunteer who once escapes exposure to infection should be ultimately proved susceptible to one or other of the species of trypanosome under examination. If this is not done it is impossible to detect an abnormally resistant subject, if indeed such exist.

In the experiments now in progress on the prophylactic effects of Bayer the position is more involved. Here it is obvious that when the effects of the drug are wearing off abnormal or subliminal infections might occur, in which demonstration of the parasite might be very difficult. Especial care is, therefore, necessary. The practical value of the results of this investigation is, however, so great that in the absence of any serious objection it is considered justifiable to continue, on the assumption that when infection does arise it will be detected. Meantime, every precaution is being taken not to overlook the presence of trypanosomes in any of these "protected" volunteers.

II. On measures of control

In any prolonged investigation of African mammalian trypanosomes, especially where Glossinae are used as agents of infection and ruminants as the vertebrate host, it is of the first importance to establish an efficient system of control against accidental infection with trypanosomes other than those which it is intended to investigate. Research of this kind has long been in progress at Entebbe, and from time to time in previous publications reference has been made to measures of control (Duke, 1921).

During the last year or so the main subject of research at Entebbe has been and still is the study of game animals, especially antelope, as a reservoir of the trypanosomes of man, and in such an inquiry supreme importance attaches to whether a given trypanosome is or is not pathogenic to man.

To carry out this research clean antelope were collected at the Institute, some of which were set aside as controls. Some months ago one of these control antelope was found to be infected with a trypanosome indistinguishable on subsequent examination from the *T. rhodesiense* carried by its experimentally infected companions.

Now it is obvious that in an investigation devised to determine the length of time which a trypanosome can retain its power of infecting man it is essential to make sure that no strain of *T. brucei* be introduced unawares. Such a strain might creep in through the agency of infected tsetse, wild or captive; or in an antelope infected before it reached the laboratory; or it might be conveyed by some biting insect other than tsetse from some animal experimentally infected with *T. brucei* at the laboratory. When, therefore, this control reedbuck became accidentally infected with a polymorphic trypanosome, it was necessary to try and find out how it acquired its infection.

In the antelope enclosure, living freely together, there are three adult bushbuck, four young adult oribi, and an adult and two young situtunga (one conceived and born at the Institute), four reedbuck (one adult and three quarter-grown animals of which the last three arrived together), and an ntalaganya. Of these three were set aside at the beginning of the investigation as controls, viz. a young situtunga, the ntalaganya and one of the young reedbuck. Since its birth at the Institute some 6 months ago, the situtunga has also been examined on frequent occasions by means of stained thick blood films. On August 24th, 1933, the control reedbuck (No. II) was found to be infected. Of the other two young reedbuck, one (No. III) had been experimentally infected with *T. rhodesiense* on July 3rd, and the other (No. IV) with Strain Tinde III *T. rhodesiense* on August 15th, 1933. All the other antelope, save one oribi and the adult situtunga, had already been experimentally infected with *T. rhodesiense* shortly after its isolation from man. The oribi and the situtunga were infected with *T. gambiense*. Evidence of continued infection after a number of months has been obtained recently in all of the antelope save two, viz. the adult situtunga and adult reedbuck, both of which appear to have lived down their infection for some time past.

The other two original control animals, together with the youngest situtunga, have remained "clean." No animal in the enclosure has been experimentally exposed to *T. brucei* at the laboratory, and as will be seen below there is no outside source of *T. brucei* anywhere near. In the monkey park there are never more than two animals and often none at all carrying *T. brucei*. The occasional strains of this trypanosome under investigation at the Institute are maintained in small rodents, in a house widely separated from both monkeys and antelope.

To consider, then, first of all the possibility of this control reedbuck being already infected when it reached the laboratory. All the reedbuck and oribi came from the Eastern Province of Uganda, from a tsetse-free area where there is no trypanosomiasis, *either of cattle or of man*. This description is based on the written testimony of the District Commissioner, the missionaries and the Veterinary Officer of that area. The Veterinary Pathologist at Entebbe has also received from the same district a number of game animals, blood films from all of which were examined for 28 days after arrival without any evidence of infection being detected. The oribi and the three young reedbuck all arrived at Entebbe at the same time; they were all unweaned and were fed from the bottle for some 6 weeks after their arrival. Thick stained films of their blood were examined on several occasions and no trypanosomes were detected. The adult reedbuck came as a kid from the same area more than a year earlier, and on arrival had its blood inoculated into several clean animals, none of which became infected.

The introduction of wild *T. brucei* into the bushbuck and the situtunga can also definitely be excluded, for on their first arrival the blood of all these animals was examined carefully both by subinoculation and by the microscope.

Moreover, they all came from the country north of Entebbe where there is no tsetse.

Everything points, therefore, to this control reedbuck being clean on its arrival at Entebbe.

And now to proceed to a consideration of the possibility that reedbuck II was infected by biting flies, tsetse or otherwise, other than those of the actual experiments.

The experimental transmission of trypanosomes by *Glossina palpalis* at Entebbe has been carried out during the last 14 years at three different laboratories. The first, on a low bluff above the pier, a few yards from the lake edge; the second at Old Entebbe about half a mile from the water's edge; and finally, since January, 1932, in the present Institute.

At the first site, wild *G. palpalis* was a rare visitor to the laboratory enclosure; at the second, such wild visitors never reached the laboratory, although *G. palpalis* could be caught along a stretch of forested lake shore about three-quarters of a mile in a direct line from the building, but well off all beaten tracks. At the first two of these laboratories special control monkeys were set aside in order to see whether casual infection ever did occur. In addition, numbers of experimental monkeys have been examined daily by the study of stained thick blood films and in the course of some 12 years no single instance of casual infection was even suspected.

The present Institute is situated on a bare hilltop 250 ft. above and about half a mile distant from the lake shore, along which for miles there is no established tsetse. The site was originally chosen as being safe from wild fly. Below the laboratory hill a canoe ferry crosses an arm of the lake about a mile wide to a cleared landing on the other side. There is no regular motor traffic down to this ferry, only occasional cars taking amateur yachtsmen to the two or three small boats that are moored near the ferry. The cars return from the lake as a rule about sunset or after. The Europeans who use these boats during week-ends are agreed that it is only rarely that tsetse are met with on the Entebbe side of the bay, which is lined for miles with papyrus behind which is no shelter for the fly. On the opposite side *G. palpalis* is to be found, though it is rare to meet fly in the ferry canoes. In the past, numbers of wild *G. palpalis* from round the Entebbe peninsula have been caught and fed on clean monkeys, but never has any polymorphic trypanosome been recovered from this or any other wild biting fly anywhere along this shore-line. The wild tsetse have an ample and varied food supply in hippopotamus, crocodile, *Varanus* and man, and *Trypanosoma grayi* is the only flagellate inhabitant of the intestine of wild flies ever recorded in this area.

There is no reason to suppose that wild *Glossina palpalis* ever come up the hill to the laboratory. The few flies that may from time to time cross the water have no inducement to go inland; there is no motor traffic between the lake shore and the laboratory at times when the flies are active, and there are plenty of natives and food animals along the water's edge. There is a small herd of

cattle kraaled at the base of the hill not far from the lake, but these are not affected with trypanosomiasis; they graze daily along the lake edge and their owners are expressly forbidden to allow them to approach the slopes of the hill.

At the Institute, as at the former laboratory sites, there is an ample system of control animals to detect casual infection, both in the monkey park and in the small herd of sheep. All sheep purchased for experimental purposes have their blood examined before they are used, both in stained thick films daily for one month and also by inoculation into monkeys: in neither collection has any such infection ever been recorded. These controls serve against infected wild flies and also any infected experimental flies that might escape. From time to time a laboratory-bred tsetse does escape from the pupa net or from the fly boxes in the laboratory. Almost all such fugitives are seen to escape and are at once caught on the windows. A fly that is not immediately caught tends to stay about the laboratory, where there are plenty of people to bite; there is no inducement to go further afield in search of food, and the rooms and corridors afford agreeable shelter. It is thus unlikely in the extreme that an escaped or, for that matter, a wild fly would leave a plentiful supply of stationary natives for the very problematical chance of a meal off a monkey or the more distant antelope hidden behind the walls of their enclosure. It must be remembered, too, that *G. palpalis* in nature likes hairless bodies to feed upon—hippopotamus, reptiles and man—and when these are readily available it will certainly never trouble to look for antelope. There is no other species of tsetse within 100 miles of the laboratory by road, the nearest being the scattered *G. pallidipes* east of Jinja, and between this fly-belt and Entebbe there is no direct road connection. The nearest *morsitans* belt is more than 200 miles away by road. The game tsetses are known to follow motor cars for long distances, but no vehicle from a game-tsetse belt has ever reached Entebbe without passing at least one night *en route*. Other genera of biting flies are with one exception exceedingly rare at the Institute site. *Stomoxys* alone is at all common, and is at times seen in numbers both in the main laboratory building and in the antelope house. Tabanids are very rare and I have never seen a *Haematopota*; culicine mosquitoes occur, but not in large numbers. The only likely direct transmitter with which we have seriously to reckon is *Stomoxys*. This species does not, however, attack the monkeys, nor at the laboratory does it annoy man—a sure indication that it has plenty of other food. There are no cattle on the hill, and its main food supply at the Institute is undoubtedly the antelope, to which it is at times a veritable nuisance. Attempts to eradicate it have so far proved unsuccessful, and the only remedy against the pest is hand catching off the mosquito wire in the antelope house. It is not uncommon for 50–100 flies to be caught on this wire netting in an hour's search. *Stomoxys* are almost always to be seen on the animals in the daytime, both inside the main stable building and in the enclosed runs that open therefrom.

There remains one other possible source of error, namely, that reedbuck II

was exposed to experimental infection without my knowledge owing to a mistake in identification. This possibility can be definitely excluded. All experiments with the antelope at Entebbe are carried out under European supervision. Each animal selected for fly experiments has a patch of its skin shaved so that the flies may feed easily: reedbuck No. II at the time of its infection had never been shaved. Moreover, the animals are in charge of a native who knows them intimately and who always supervises their capture. No one with experience of native herdsmen's knowledge of their animals, even when in charge of a large number, would ever entertain the possibility of a mistake in identity occurring, and in addition to their natural peculiarities by which the antelope are known to their keeper, the three young reedbuck all bear distinctive marks by which they are distinguished in the laboratory register.

I have carefully examined all obviously relevant factors that can have accounted for the infection of this control reedbuck and have been led to the conclusion that it was infected from one of its companions, almost certainly reedbuck No. III, by means of wild *Stomoxys* in the antelope enclosure. The three young reedbuck keep close together all through the day, thus facilitating the direct transference of trypanosomes from one to the other; moreover, trypanosomes are common in their peripheral blood and appear in slides day after day, whereas in blood slides from the bushbuck and the adult reedbuck for many months now no trypanosomes have been seen, and from the oribi only occasionally. Both these facts favour direct transmission from reedbuck to reedbuck. The trypanosome in reedbuck No. II, the infected control animal, behaves like *Trypanosoma rhodesiense*; it is readily transmissible by laboratory-bred *Glossina palpalis*; and flies whose glands are infected with it readily infect monkeys but cannot infect man. The gland infections of flies infected from this reedbuck are scanty and indistinguishable from the infections produced by the strain with which one of its young companions (reedbuck III) is infected, *i.e.* *Trypanosoma rhodesiense*, Strain Tinde I, which produces a very characteristic type of gland infection in *Glossina palpalis* (Duke, 1933). Strain Tinde I was tested at just about the same stage in its laboratory career that it was introduced into reedbuck III, and was found to be no longer pathogenic to man. Moreover, the trypanosomes of reedbuck III when tested in metacyclic form on man in December, 1933, and also the metacyclic forms derived from a monkey inoculated from this reedbuck by the syringe some 6 weeks earlier, were both non-infective to man. And lastly, to complete the resemblance, flies cyclically infected from a monkey inoculated from reedbuck II by the syringe also failed to infect man.

After the detection of this "flaw" infection an attempt was made to transmit *Trypanosoma rhodesiense* by the direct method from monkey to monkey by *Stomoxys*. Suffice it to say here that the clean monkey became infected. Trypanosomes were more numerous in the monkey's blood than is likely to occur in the reedbuck, nevertheless the readiness with which this attempt succeeded was remarkable. Dissection of several hundreds of *Stomoxys* caught

in the antelope stable revealed in the partially digested blood of the hind-gut of a single fly a few feebly moving trypanosomes, that had presumably been taken up from one of the antelope. One infection of *Stomoxys* with flagellates of crithidial or leptomonas type was also seen (cf. Duke, Mettam and Wallace, 1934).

The length of this discussion is, I hope, warranted by the intrinsic importance of the incident, which also illustrates well the need of extreme care in the conduct of experiments where ruminants are employed. The danger is, of course, far greater where game tsetses are within range of the scene of the experiments. Only one of the control antelope at Entebbe has become infected, and this suggests that direct transmission of the polymorphic trypanosomes from ruminant to ruminant by *Stomoxys* can only occur in specially favourable circumstances such as very close contact between animals and the presence of plenty of trypanosomes in the peripheral blood. In nature the necessary conditions might occur among gregarious species of game, and it may well be that, besides *Stomoxys*, *Lyperosia*, which occurs in incredible numbers on game in certain regions, can act as a transmitter.

There is no evidence that direct transference of trypanosomes from animal to animal occurred in any of the other antelope of the enclosure, all of which have been examined by fly tests and by blood inoculation and examination for many months. As we have seen, the three young reedbuck form a group apart. But the possibility must be borne in mind. In consequence of this episode it has been decided not to introduce any more strains into the present antelope quarters, save such as may be necessary for superinfection experiments. Then, in the very improbable event of any further circulation of *Trypanosoma rhodesiense* taking place within the kraal, the date of the introduction of the last strain, now some eight months ago, will at least fix the *minimum* period that any of these trypanosomes has resided in antelope blood.

To summarise the results of this inquiry. One of four control antelope has been infected. The trypanosome in this animal is *T. rhodesiense*, and its behaviour in the glands of *Glossina palpalis* is identical with that of the strain infecting a close and constant companion of the infected control animal. The trypanosome in this companion and that in the infected control antelope are both incapable of infecting man.

At the time when reedbuck II was found to be infected, trypanosomes were found almost daily in the peripheral blood of its companion, reedbuck III, whereby the latter differed from the other infected animals (in the kraal), in whose blood trypanosomes were rarely if ever seen after the early days of their infection. I consider it therefore justifiable to conclude that this control reedbuck was infected from its companion No. III by the agency of *Stomoxys*; and that it is exceedingly unlikely that the conditions in the kraal allow of the direct transference of trypanosomes except in such peculiarly favourable circumstances as those obtaining at that particular time among the young reedbuck. Reedbuck No. IV must also come under suspicion, and in the report on the antelope reservoir experiments both these reedbuck, Nos. II and IV, will

be dealt with separately as carrying in all probability Strain Tinde I, and in the case of No. IV probably a mixed infection of Strains I and III.

Before leaving this subject it may be noted how little evidence there is in the various reports on trypanosomiasis investigations that any serious attempt was made to exclude or even detect the occurrence of casual or accidental infection. The reader is often left to form his own conclusions on this important matter. Where trypanosome investigations are carried out in the proximity of game-tsetse country the use of domestic ruminants as clean animals is fraught with considerable risk. To give but one example, the attraction exerted on certain of the game tsetses by rapidly moving vehicles is now a matter of common knowledge, and yet it is stated in one report that cyclists were regularly employed to bring in material to the laboratory from closely adjacent fly country. In such circumstances it would be little short of a miracle if wild tsetse did not from time to time obtain access to the laboratory animals; but there is no reference to this possibility or its prevention in the text.

Of all the animals commonly employed in the investigation of man's trypanosome, monkeys are perhaps the most useful. They are relatively easily infected, even with *Trypanosoma gambiense* in its mildest form; their activity and intelligence render them less vulnerable to the attention of winged biting insects than are domestic ruminants; and they succumb fairly rapidly to the polymorphic group, whereas ruminants may remain for many months infected without showing any obvious physical signs. In a community of captive monkeys it is easy to set up a system of controls that will detect accidental infection, so that the lack of reference to any such system from so many reports is the more surprising. These comments are the outcome of many disappointments in the perusal of published work. Scientific research has not yet reached a stage of perfection when an adequate system of control can be assumed as a *sine qua non* of publication; and it is surely the reader's right to know in reasonable detail what has been done, so that he may be able to judge for himself the value of the conclusions reached.

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INSECT PARASITES OF PSYLLIDAE

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(With 4 Figures in the Text)

The insect parasites bred out of Psyllid hosts, in the course of studying their biology in Scotland, belong to four families of Hymenoptera and one of Diptera, the total number of species encountered being seven. Of these one and possibly two more of the hymenopterous species are hyperparasites. The hosts involved include four species, of which only one, *Psyllia mali* race *peregrina*[1], may be said to have been fairly heavily parasitised. As observed by Ferrière (1926), species of Psyllidae, in spite of their nymphs leading a comparatively sedentary life, are less susceptible to attack by parasites than are the other Homoptera. As a rule the hymenopterous parasites attack them in the nymphal stage, while the dipterous species parasitise the adults as well.

Waterston (1922) has given a list of Chalcid parasites of Psyllidae with descriptions of some of them. Since then some new records of Chalcid and other parasites have been made, and they are listed here (Tables I, II and III), with the omission only of those already mentioned in Waterston's paper.

Parasites of *Psyllia mali* Schmidberger race *peregrina*

Life history of the pest, Psyllia mali *race* peregrina

The adults appear about the end of May and are found in the field till the first week of October. Eggs which are laid in the latter part of autumn, overwinter and hatch early in April. The nymphs creep to the half-opened leaf buds and feed gregariously in the first two instars. Later they distribute themselves over the undersurface of the leaves and on the petioles, where they feed singly and secrete little tubes of white waxy substance. The nymphal life lasts for about seven weeks, and nymphs of the last instar become abundant in the field about the middle of May. The adult insects are at first small (2·5 mm. in length) and green; later they are more robust and brownish, and even reddish. It was observed that the colour of the males changed earlier than that of the females. Mating starts in the last week of August, and eggs are laid on the twigs of the host plant, a few days afterwards, till the middle of October, when the adult insects die. Hawthorn is the sole food plant, and it is not possible to rear this race successfully on apple.

[1] This species has, so far, been recognised as *Psyllia peregrina* Först. In another paper (1934) I have given reasons for regarding it as a biological race of the apple sucker, *P. mali* Schmidberger.

Table I. *Parasites attacking nymphs.*

Parasite species	Family and superfamily	Host species	Country	Reference
1. *Pachyneuron validium*	Pteromalidae (Chalcidoidea)	*Euphyllura arbuti* Schwarz	California	Waterston, 1923
2. *Pachyneuron* sp.	,,	*Psyllia mali* Schmidberger race *peregrina*	Scotland	Present paper
3. *Asaphes vulgaris* Walk.	,,	,,	,,	,,
4. *Pteroptrix maskelli* Ashm.	,,	*Eurhinocola eucalypti* Maskell	New Zealand	Gourlay, 1930
5. *Prionomitus mitratus* Dalm.	Encyrtidae (Chalcidoidea)	*Psyllia pyrisuga* Först.	France	Ferrière, 1926
6. ,,	,,	*P. retamae* Pub.	Spain	Mercet, 1926
7. ,,	,,	*P. mali* Schmidberger race *peregrina*	Scotland	Present paper
8. *Cercobelus jugoeus* Walk.	,,	*Psyllopsis fraxinicola* Först.	,,	,,
9. *Psyllaephagus arbuticola* Gahan and Waterston	,,	*Euphyllura arbuti* Schwarz	California	Gahan and Waterston, 1926
10. *P. euphyllurae* Silv.	,,	*E. olivina* Costa	Italy, Sicily, Portugal	,,
11. *P. iwayensis*	,,	Psyllid sp. on *Cinnamomum*	Japan	Ishi, 1928
12. *P. phytolymae*	,,	*Phytolyma lata* Scott	Nigeria	Ferrière, 1931
13. *Psyllaedontus viridiscutellatus*	,,	Psyllid sp. on *Elaegnus*	—	Ishi, 1928
14. *Aprostocetus roseveari*	Eulophidae (Chalcidoidea)	*Phytolyma lata* Scott	Nigeria	Ferrière, 1931
15. *Platygaster* sp.	Platygasteridae (Proctotrypoidea)	*Psyllia mali* Schmidberger race *peregrina*	Scotland	Present paper

Table II. *Parasites attacking adults. Order Diptera: family Cecidomyidae.*

Parasite species	Host	Country	Reference
1. *Endopsylla agilis* de Meijere	*Psyllia försteri* Flor.	(i) England; (ii) Holland	(i) Bagnall and Harrison, 1924; (ii) Barnes, 1930
2. *Endopsylla* sp.n.	*P. mali* Schmidberger *P. pyricola* Först. *P. melanoneura* Först.	Scotland	Present paper
3. *Lestodiplosis liviae** Rubo	*Livia juncorum* Latr.	Germany, England	Barnes (*loc. cit.*)
4. *Lestodiplosis* sp.	—	Europe	,,
5. *Bremia* sp.	*Psyllopsis fraxini* Lin.	,,	,,

Table III. *Hyperparasites attacking primary Encyrtid parasites.*

Hyperparasite	Family and superfamily	Host (primary parasite)	Country	Reference
Lygocerus semiramosus Kieff.	Caliceratidae (Proctotrypoidea)	*Prionomitus mitratus* Dalm.	Scotland	Present paper

* This species is also recorded by Bagnall and Harrison (*loc. cit.*) as living as inquiline in the gall of *Livia juncorum* on *Juncus* sp. in Durham, England.

Parasites attacking nymphs

The following hymenopterous species were reared in the laboratory from parasitised nymphs brought from the field:

(1) *Prionomitus mitratus* Dalm. (Encyrtidae: Chalcidoidea).
(2) *Platygaster* sp. (Platygasteridae: Proctotrypoidea).
(3) *Lygocerus semiramosus* Kieffer (Caliceratidae: Proctotrypoidea).
(4) *Asaphes vulgaris* Walk. (Pteromalidae: Chalcidoidea).
(5) *Pachyneuron* sp. (Pteromalidae: Chalcidoidea).

Of these, *Prionomitus mitratus* was fairly common, parasitising 20–30 per cent. of the nymphs. It has been reported by Ferrière (1926) as a parasite of *Psyllia pyrisuga* Först. and by Mercet (1926) as a parasite of *Psyllia retamae* Pub. Other species of the genus have been known as parasites of Coccids[1].

Parasitism by species of Platygasteridae was very rare. *Lygocerus semiramosus* is a hyperparasite of *Prionomitus mitratus*. The last two species, *Asaphes vulgaris* Walk. and *Pachyneuron* sp., are considered by Dr Ferrière, to whom the insects were submitted for identification, as hyperparasites, as they have also been bred from parasites of aphids. Two other species of *Pachyneuron* have been recorded by Waterston (1922, 1923) as parasites of Psyllidae: *Pachyneuron crassiciume* Waterston parasitising *Rhinocola populi* Laing, the Psyllid attacking *Populus euphraticus* in Mesopotamia, and *P. validium* Waterston parasitising *Euphyllura arbuti* Schwarz in California.

Parasite, Prionomitus mitratus

Parasitised nymphs of *Psyllia mali* race *peregrina* occur in the field from the first week of June to the middle of September, and may be recognised by their bloated appearance and deep brown coloration. The assumption of brown colour by the nymphs marks a late stage in the development of the parasite, when the viscera of the former has been almost completely devoured by the parasitic larva, which then pupates in a membranous sheath inside the host's body. Husain and Nath (1923) have recorded a similar change in the colour of the nymphs of *Diaphorina* (*Euphalerus*) *citri* Kuw. when parasitised by *Tetrasticus radiatus* Waterston (Eulophidae). The Encyrtids emerge through a round hole in the dorsum of the abdomen; the exit is seldom made on the ventral side. A few days before emergence the black body of the parasite is apparent through the integument of the host, and the head of the former is always turned towards the posterior end of the latter. The first batch of parasites appeared in the laboratory on 29. vi. 33. The adult insects are black and shiny and have a pronounced sexual dimorphism in the antennae, the

[1] Members of an allied genus, *Psyllaephagus*, have also been known to parasitise many species of Psyllidae, and the two are so alike in biology and morphology that they have sometimes been regarded as synonymous. This is especially the case because of the difficulty of separating the females of the two genera, the males being distinguished chiefly by the long hairs of the antennae in *Prionomitus* and the short hairs of the antennae in *Psyllaephagus*. This view, however, is not upheld by Mercet (*loc. cit.*), who has given characters differentiating the two genera.

flagellar segments of which, except the last one, are triangular and hairy in males, and rounded and without hairs in females. Many specimens of *Prionomitus mitratus* were beaten from coniferous trees, growing in the vicinity of ash and hawthorn, in November and December, and, hence, it is possible that they pass the winter in the adult stage.

Hyperparasite, Lygocerus semiramosus *Kieffer*

From the parasitised nymphs adults of *Lygocerus semiramosus* were also reared. These were believed to be hyperparasites of *Prionomitus mitratus*. They appeared towards the middle of September, and considering the fact that three of their egg shells with the larvae just hatched were dissected from a nymph on 28. ix. 32, it may be presumed that they were acting as tertiary parasites of a secondary parasite or of their own species. A parallel instance of hyperparasitism has been recorded by Haviland (1920) in the case of the quaternary *Lygocerus cameroni* Kieffer attacking a tertiary Chalcid or a Cynipid which was parasitic on a primary Chalcid parasitic on *Aphidius ervi*, a Braconid which was parasitising an aphid, *Macrosiphum urticae*.

The egg of *Lygocerus semiramosus* (Fig. 1) is long elliptical, 0·67 × 0·28 mm., the whole surface being sculptured with fine longitudinal striae. The larva emerges through an opening at the anterior end, at which an operculum separates from the remainder of the egg. The larva is a small grub, creamy white in colour and measuring 0·45 × 0·15 mm.

The male adult insect was described by Kieffer (1907, 1914).

The female (Fig. 2 A) measures 1·3 mm. in length and its antennae differ from those of the male (Fig. 2 B) both in shape and in the absence of long hairs. The basal segment of the latter is 0·27 mm. long and stout, followed by the ten small, somewhat cylindrical segments of the flagellum, gently curved as a semicircle. The terminal segment is twice as long as others preceding and measures 0·11 mm.

Various species of *Lygocerus* have been recorded as parasites or hyperparasites of aphids and other Homoptera. *L. semiramosus* was recorded by Kieffer (*loc. cit.*) from Scotland and France, but its hosts were unknown.

Parasite attacking adult

Parasites of adult Psyllidae have been recorded only from the dipterous family Cecidomyidae (see list on p. 326), and Speyer (1929) recently recorded an unidentified Cecid species parasitising *Psyllia mali* (apple form), figuring the egg and the apparently full-grown larva. In this region the Cecid parasite discovered proved to be a new species[1] of the genus *Endopsylla*, and its hosts were *Psyllia mali* Schmidberger race *peregrina*, *P. mali* race *mali*, *P. melanoneura* Först. and *P. pyricola* Först. The first-named race of *P. mali* was the most heavily parasitised, and observations recorded here refer chiefly to this

[1] This species is being described from the material sent by me to Dr H. F. Barnes of the Rothamsted Experimental Station.

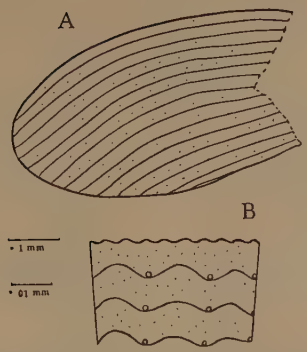

Fig. 1. A, egg shell of *Lygocerus semiramosus* Kieffer. B, a portion of the same magnified.

Fig. 2. *Lygocerus semiramosus* Kieffer. A, female adult. B, antenna of the male.

species as host. The percentage of parasitism in *P. mali* race *peregrina* in this locality may be judged from the following tabulated counts made on random collections:

Date	Number of adults examined	Number of adults parasitised	Percentage
13. ix. 32	79	32	40·5
13. vi. 33	67	14	20·8
26. vi. 33	68	15	22·5
30. vi. 33	57	13	22·8
21. vii. 33	137	28	20·4
4. viii. 33	24	9	37·5

Observations showed that the incidence of parasitism was higher in female Psyllids than in males.

Effect of parasite on host

The first symptoms of the presence of the parasite appear when the parasitic larva pierces the abdomen of the host and passes into the haemocoele. The Psyllid gradually becomes more and more sluggish until it loses all power of jumping and the abdomen becomes swollen as a result of the growing larva inside. As the parasitic larva develops during the egg-laying season of *P. mali*, the swollen abdomen of the host may well be mistaken for that of a gravid female. In female hosts the ovarian eggs may develop, up to a certain stage, simultaneously with the parasitic larva. A Cecid larva and twelve mature eggs were found in a female on 13. ix. 32. Healthy females showed as many as twenty-two mature eggs at this period. When the larva is ready to emerge for pupation, however, no trace of eggs or viscera is left in the moribund Psyllid body. Unlike their nymphs, the parasitised adults do not undergo any change of coloration due to the presence of the parasite.

Biology of *Endopsylla* sp.n.

Egg. Very small, oval with a minute basal stalk, 0·17 × 0·06 mm.; chorion smooth.

Eggs are laid singly on the fore-wings of the host, their basal stalk being inserted in the wing membrane alongside one of the veins. As a rule one egg, rarely two or three, is deposited on one or other wing of the host, seldom on both (Fig. 3 E). They are pale yellowish white turning deep yellow near hatching, when the two red eyes of the larva shine through the chorion. Eggs were first noticed early in June and may be found in the field as late as the middle of August.

Larva. The larvae hatch in about 8–13 days and then crawl from the wings to the body of the host. There they usually feed for 3–4 days as ectoparasites, and then burrow through one of the intersegmental membranes into the haemocoele of the Psyllid. They remain inside for 6–10 days, devouring the viscera of the host till they are well-developed maggots. They then pierce the host abdomen at its base and make their way out again. They crawl and live a day

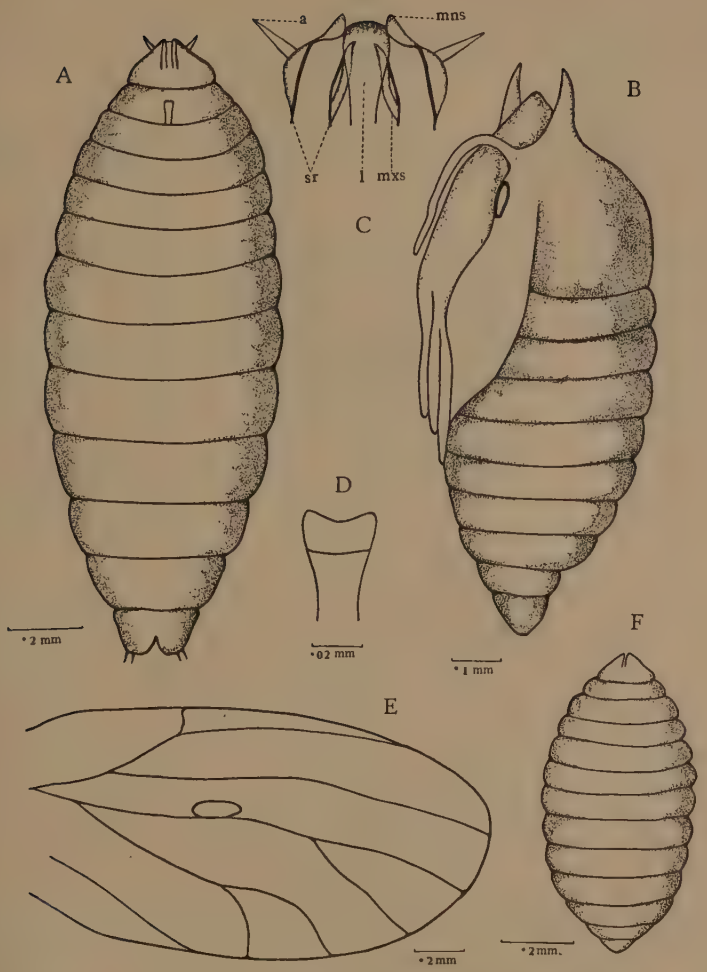

Fig. 3. *Endopsylla* sp.n. A, larva (advanced stage). B, pupal skin. C, mouth-parts of advanced larva. D, sternal spatula. E, forewing of *Psyllia mali* race *peregrina* with an egg of *Endopsylla* sp.n. F, larva (early stage).

Lettering:

a, antenna; *l*, labium; *mns*, mandibular stylet; *mxs*, maxillary stylet; *sr*, skeletal rods.

or two on hawthorn leaves and then drop to the ground to pupate. Three stages of the larvae could be distinguished:

First stage. Just before burrowing into the host abdomen; 2 or 3 days old. Creamy yellow grub, 13-segmented including the cephalic segment. 0·47 × 0·21 mm.

Second stage (Fig. 3 F). Dissected from host abdomen. Differs from the previous larva in its larger size and more pronounced oral aperture carried on a minute papilla. 0·90 × 0·45 mm.

Third stage (Fig. 3 A). Larva just emerged from host abdomen, prior to pupating. Creamy yellow, often with deep green or orange pigmented material shining through integument. Differs from previous larva in possessing a pair of small single-segmented antennae, a well-developed retractile oral papilla and a sternal spatula in the postcephalic segment. The mouth-parts (Fig. 3 C) are well formed (being used to cut through the integument of the host) and consist of a labium, a pair of slender but sharply pointed maxillary stylets, a pair of stout forwardly projecting mandibular stylets, a labrum and two pairs of heavily sclerotised skeletal bars running posteriorly. Anal segment slightly notched in middle, with two small setae each on a small protuberance on either side. 1·90 × 0·64 mm.

Pupa (Fig. 3 B). Just before pupation the larva starts weaving a fibrous case round itself, which later takes the shape of the adult insect and is shed like a moulted skin after its emergence. The pupal stage lasts for about 6 days in early autumn, and the adult emerges by rupturing the pupal skin anteriorly with its fore-legs and pulling itself out by convulsive movements of the body.

Adult (Fig. 4 A). The first adults, reared in the laboratory, appeared in the first week of July. As the eggs are found long after this period, it is possible that a second generation also occurs, although I have no definite evidence for this. The adult Cecids are of bright orange colour, with blackish brown eyes, and are very active. They were invariably observed to be attracted towards the light. The insects are characterised by marked sexual dimorphism of the antennae. In both sexes, the two basal segments are cup-shaped, one fitting into the other. In the male antennae (Fig. 4 B) each segment other than the basal and the terminal consists of two bulbous portions, the one a little different in shape from the other, and two tubular constrictions. Besides numerous minute pointed structures, the proximal bulbous portion is ornamented with a whorl of arched filaments ("filets arqués" of Kieffer, 1913) and the distal bulbous portion with two similar whorls. In the female antennae (Fig. 4 F and G) each segment, except the two basal and the terminal, has only one elongated bulbous part and a tubular constriction following it, and the arched filaments are replaced by small bristles. In both sexes the antennae are 12-segmented. The male adult measures 1·2 mm. in length and the female 1·4 mm.

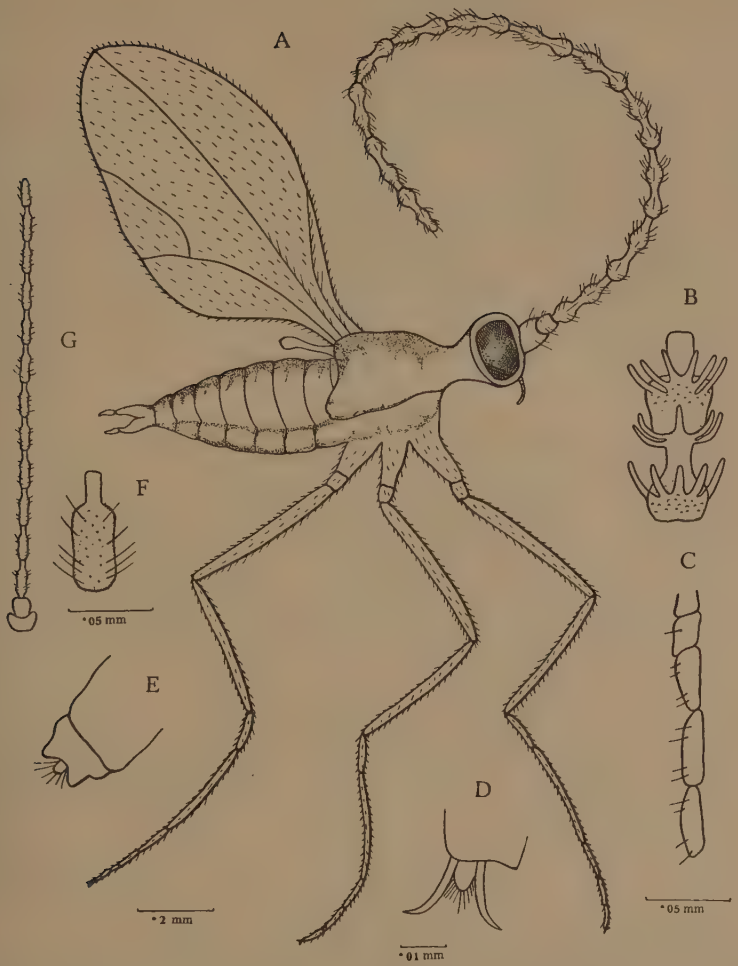

Fig. 4. *Endopsylla* sp.n. A, male adult. B, a segment of the antenna of the male. C, maxillary palp. D, apex of tarsus. E, terminal segments of the female abdomen. F, a segment of the antenna of the female. G, female antenna.

Parasites of *Psyllopsis fraxinicola* Först.

The only parasite bred from the nymphs of this Psyllid was an Encyrtid, *Cercobelus jugoeus* Walker, a very rare species and known only from the British Isles, although its host was previously unknown. Parasitism due to this species was neither high nor widespread, and the parasitised nymphs showed all the symptoms exhibited by the nymphs of *Psyllia mali* race *peregrina* when similarly attacked by other Encyrtids. The parasitised nymphs

were collected in the field in August at Boghall, Midlothian, and Dalkeith, Midlothian.

ACKNOWLEDGMENTS

I wish to express my grateful thanks to Dr A. E. Cameron, for advice given during the progress of the work and for much helpful criticism in the preparation of the manuscript. My thanks are also due to Sir Guy A. K. Marshal and Dr Ch. Ferrière for the identification of the hymenopterous parasites and to Dr H. F. Barnes for the identification of the Cecidomyid parasite. I am also indebted to the Imperial Institute of Entomology, especially to Dr S. A. Neave, for the loan of reference literature not available in Edinburgh. Lastly I wish to acknowledge gratefully a grant made by the Trustees of the Moray Endowment Fund, Edinburgh University, which helped to defray part of the expenses of this work.

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THE STATUS OF SPECIES OF *TRICHOSTRONGYLUS* OF BIRDS

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(With 13 Figures in the Text)

Historical review

Two species of Nematodes belonging to the genus *Trichostrongylus*, namely, *T. tenuis* (Mehlis 1846) Railliet and Henry 1909, and *T. pergracilis* (Cobbold 1873) Railliet and Henry 1909, have been described from birds, the location of both species being the caeca. A third species, described as *Strongylus douglasi* Cobbold 1882 emend. Gedoelst 1911, from the proventriculus of the ostrich, is considered by some authors as belonging to the genus *Trichostrongylus*, and by others, notably Lane (1923) and Nagaty (1932), as necessarily referred to a distinct genus, *Libyostrongylus*, with which latter opinion we are inclined to agree, as a result of examination of specimens of this species.

Trichostrongylus tenuis, originally described by Mehlis (1846) from the ring-necked pheasant in Europe, was subsequently recorded from the duck, goose, chicken, turkey and European partridge; records of its distribution have been mainly European (British Isles, France, Germany, and U.S.S.R.), but there has also been one report each, up to the present time, from Asia (Russian Turkestan), Africa (Union of South Africa) and North America (United States (District of Columbia)).

T. pergracilis was originally described from the red grouse of England; Cobbold (1873), however, when describing the species, made no reference to the already existing species, *T. tenuis*, and there is, therefore, the possibility that it was overlooked by him. Cobbold's description, including his illustrations, of *T. pergracilis* is so meagre of detail as to be indistinguishable from that of *T. tenuis*, but subsequent descriptions, especially those of Shipley (1911) and Cram (1927), included for *T. pergracilis* characteristics which did not conform with those described for *T. tenuis*. The only records of *T. pergracilis* are from grouse of the British Isles and bob-white quail of the United States.

In recent years grave doubts have arisen as to the constancy of the differences between the two species and, therefore, as to the validity of the second species, *T. pergracilis*. Nagaty (1932) has indicated that he considers the two species as *probably* identical, which opinion we have also held for some time.

Present investigation

With the recent finding[1] of a species of *Trichostrongylus* in two localities and in two bird hosts, namely, ring-necked pheasants in Michigan and domestic geese in the District of Columbia, we were confronted with the necessity of deciding on their specific identity. The existing descriptions being inadequate for a critical differentiation of *T. tenuis* and *T. pergracilis*, it was concluded that a comparison of our material with specimens from grouse and from partridges of Great Britain was highly desirable.

Through the courtesy of E. L. Taylor of the Ministry of Agriculture and Fisheries, Weybridge, England, we were supplied with a large number of specimens of *T. pergracilis* collected from grouse in Scotland. Our material, collected from pheasants and geese, was found to be indistinguishable from the grouse material.

Subsequently, through the courtesy of W. E. Collinge, Keeper of the Yorkshire Museum, York, England, who had recently (1932) completed an investigation of diseases of the partridge in England, and had designated *T. tenuis* as of frequent occurrence, a generous supply of those nematodes was made available to us. A comparative study of these specimens with the material referred to above convinced us that only one species was involved and that the morphological differences which had previously been considered of specific value for *T. tenuis* and *T. pergracilis* were not consistent and were therefore not significant.

Additional material from the collection of the Zoological Division was also examined; the comparative observations made by us were, therefore, based on trichostrongyles from the following localities and hosts:

United States: pheasant (*Phasianus colchicus*), blue goose (*Chen caerulescens*), Canadian goose (*Branta canadensis*), domestic goose (*Anser anser domesticus*), guinea-fowl (*Numida meleagris*), chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*), and bob-white quail (*Colinus virginianus*).

Great Britain: red grouse (*Lagopus scoticus*) and European partridge (*Perdix perdix*).

The conclusion arrived at by us was that only one species was involved, and that it is, therefore, justifiable to consider *T. pergracilis* synonymous with *T. tenuis*. As evidence to that effect may be mentioned the following observations.

Nagaty (1932), in a review of the species of the genus *Trichostrongylus*, stated that *T. pergracilis* and *T. tenuis* were very similar, and remarked that the only handicap that kept him from deciding whether these species were one and the same or two distinct species was the scarcity of good material of *T. tenuis*. The only difference he could detect between the two species was the slightly larger size of *T. pergracilis*. The matter of difference in size, provided the two species were morphologically identical, he believed could be explained

[1] Biological and pathological phases of these infestations are to be discussed in a paper by Cram and Cuvillier, to be published in the same number of this *Journal*.

on the grounds that the parasite flourishes better in the grouse than in other birds.

The present writers were able to find a number of individual specimens of *T. tenuis* from the partridge which were just as long as the longest recorded specimens of *T. pergracilis* from the red grouse, although on the whole specimens from the partridge were, on the average, slightly smaller than those from the grouse. The material from the chicken, turkey and guinea-fowl was that obtained by Cram and Cuvillier by feeding these birds under experimental conditions with trichostrongyle larvae hatched from eggs of nematodes originally obtained from pheasants. The average length of the specimens in these three birds, and also of those in geese, showed considerable variation; it is, therefore, considered untenable to view size as a distinguishing characteristic.

Nagaty failed to find differences in the dorsal ray of the bursa, as regards the splitting of the ray into branches of greater or less length, and also the division or non-division into two lobules of the small median lobe of the bursa, which differences had been shown in the figures by Railliet (1893) and by Cram (1927). Our comparative studies revealed considerable variation in these characters, among specimens of the same host origin, and we can, therefore, no longer consider them valid specific differences.

In accordance with the present evidence, the description of *T. tenuis* must be emended to include those characters and measurements formerly credited to *T. pergracilis*.

Trichostrongylus tenuis (Mehlis 1846) Railliet and Henry 1909.

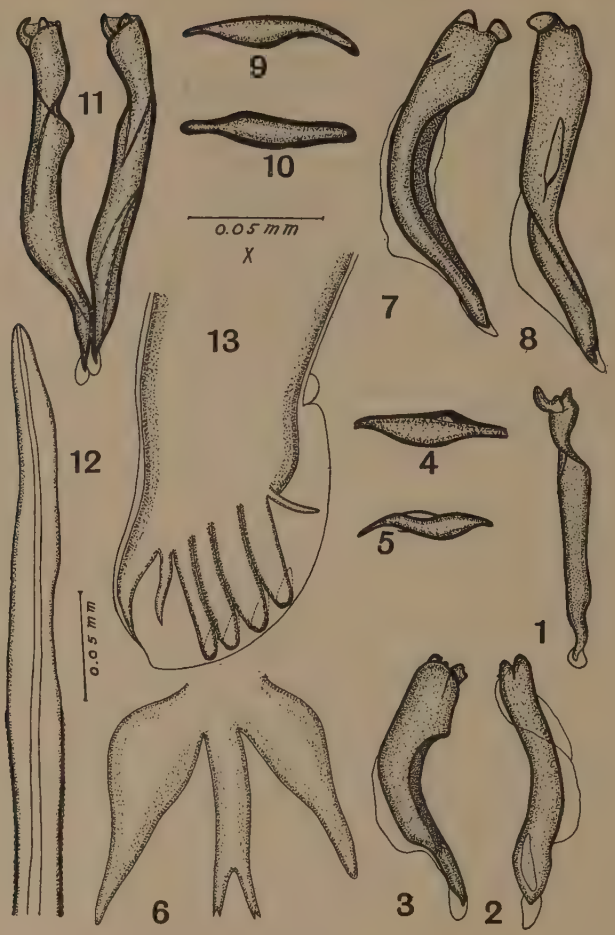
Synonyms. Strongylus tenuis Mehlis 1846 (in Creplin, 1846); *S. pergracilis* Cobbold 1873; *Strongylus serratus* Linstow 1876; *Trichostrongylus pergracilis* (Cobbold 1873) Railliet and Henry 1909.

Hosts. Anas platyrhynchos, A. p. domesticus, Anser albifrons, A. anser, A. a. domesticus, Branta canadensis, Chen caerulescens, Gallus gallus, Lagopus scoticus, Meleagris gallopavo, Numida meleagris, Otis tarda, Perdix perdix, Phasianus colchicus.

Location. Caeca and, less frequently, small intestine.

Morphology. Trichostrongylus: worms small and slender. Body gradually attenuated in front of genital opening. Oral opening more or less triangular, surrounded by three small, inconspicuous lips. Four submedian cephalic papillae and two amphids present. Cuticle of anterior end of body lacking conspicuous transverse striations for a distance of about 200–250μ from extremity, then with distinct serrated appearance ("sägeförmig," as described by Linstow (1876)) for a distance of about 1·2 mm. or more. Head 12–14μ wide. Buccal cavity very weakly developed. Oesophagus relatively long, 693–824μ, simple, gradually thickening toward posterior end. No cervical papillae (deirids).

Male. 5·5–9 mm. long by 48μ wide near centre of body; maximum width 61–100μ, just anterior to bursa. In specimen 8·5 mm. long, 48μ wide near centre of body, nerve ring about 141μ from anterior end. Cuticle inflated on ventral surface just anterior to bursa. Dorsal lobe of bursa not distinctly marked



Figs. 1–13

Figs. 1–6. *Trichostrongylus tenuis* from European partridge. 1, right spicule, ventral view; 2, right spicule, ventro-lateral view; 3, right spicule, lateral view; 4, gubernaculum, dorsal view; 5, gubernaculum, lateral view; 6, dorsal and externo-dorsal rays of bursa, showing variation which may occur in length of latter. Camera lucida drawings.

Figs. 7–11. *Trichostrongylus tenuis* from red grouse. 7, right spicule, lateral view; 8, left spicule, lateral view; 9, gubernaculum, lateral view; 10, gubernaculum, dorsal view; 11, right and left spicules, ventral view. Camera lucida drawings.

Fig. 12. *Trichostrongylus tenuis* from partridge. Anterior extremity of male showing conspicuous striations on cuticle beginning at some distance back of head. Camera lucida drawing.

Fig. 13. *Trichostrongylus tenuis* from partridge. Bursa, lateral view. Semi-diagrammatic. Scale *X* refers to Figs. 1–11.

off from lateral lobes; in dorsal view of bursa, width of dorsal lobe indicated by a slight indentation in margin. Each lateral lobe supported by six rays, the tip of the ventro-ventral directed slightly ventrad, and the externo-dorsal directed slightly dorsad. Latero-ventral and externo-lateral about equal in width, both slightly wider than either the medio-lateral or the postero-lateral rays; medio-lateral ray slightly wider than postero-lateral ray. Externo-dorsal ray broad at base and gradually narrowing towards apex, usually shorter than dorsal ray, the latter 44–66μ long (except in occasional specimens where sometimes poorly developed and only 33–36μ long), bifid at its distal third, and each of these divisions again bifid and very finely pointed. Spicules dark brown in colour, slightly unequal in length, the left 120–164μ long, the right 104–150μ long; both much twisted, especially at distal ends, and provided with an ear-like structure on proximal end. In ventral view, right spicule (Figs. 1, 11) very much constricted laterally just posterior to proximal region, this constriction not evident in lateral view. In lateral view (Figs. 3, 7), this same spicule presenting an indentation just back of the proximal region on the concave side. Both spicules apparently surrounded in distal two-thirds by a thin membrane extending for a short distance beyond distal ends. Gubernaculum (Figs. 4, 5, 9 and 10) 60–83μ long, strongly cuticularised along margins. In ventral and dorsal views, gubernaculum spindle-shaped, with an anterior long narrow portion and a posterior short broad portion. A slight protuberance just at base of long narrow anterior portion on right side.

Female. 6·5 mm.–1·1 cm. long by 77–100μ wide at level of vulva. Nerve ring about 141μ from anterior end of body in specimen 9·5 mm. long. Vulva 750μ to 1·9 mm. from posterior end of body, opening slit-like, 57–70μ long, with crenulated edges. Uteri divergent. Ovejectors well developed, their combined lengths from 308 to 462μ. Anus 77–120μ from tip of tail. Intra-uterine eggs thin-shelled, elongated, 65–92μ by 35–46μ.

Distribution. Africa (South Africa (Natal)), Asia (Russian Turkestan), Europe (France, Germany, Great Britain, U.S.S.R.), and North America (United States).

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OBSERVATIONS ON *TRICHOSTRONGYLUS TENUIS* INFESTATION IN DOMESTIC AND GAME BIRDS IN THE UNITED STATES[1]

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Trichostrongylus tenuis (Mehlis 1846) Railliet and Henry 1909, was originally described from Europe; it was subsequently collected in Asia, Africa and North America, but until the present report it has not been recorded as a disease producer outside Europe. There have recently come to light outbreaks of disease in anseriform and galliform birds in the United States, associated with the presence of large numbers of this strongyle in the caeca, and material derived from these cases enabled the writers to conduct some studies on life history, cross-transmission and pathology.

Previous investigations of avian trichostrongylosis

In 1873, reporting his investigation of an epidemic of disease among red grouse (*Lagopus lagopus scoticus*) of Great Britain, Cobbold (1873) expressed the opinion that two species of parasites, namely, the tapeworm *Taenia calva* Baird 1853 (synonym of *Raillietina urogalli* (Modeer 1790) Fuhrmann 1926) and a Nematode of the group of strongyles, both found frequently in the diseased grouse, were of etiological significance. Cobbold, failing to make any mention of the strongyle previously described from the ring-necked pheasant, namely, *Trichostrongylus tenuis*, described his specimens from the grouse as a new species, *Strongylus pergracilis* (synonym of *Trichostrongylus pergracilis* (Cobbold 1873) Railliet and Henry 1909). A subsequent investigation, reported by Shipley (1911) in 1911, revealed this same Nematode as almost universally present in some two thousand red grouse examined in Great Britain, during outbreaks of severe disease among the birds.

Concerning the effect of the species *T. tenuis* on bird hosts, a partridge disease inquiry, conducted in England during the years 1931 and 1932 and reported by Collinge (1932), found that trichostrongylosis, caused by *T. tenuis*, was prevalent in epizootic form among the partridges (*Perdix perdix*).

It now appears (Cram and Wehr, 1934) that *T. pergracilis* is synonymous with *T. tenuis* and, therefore, that the same species of nematode is present in grouse disease and in partridge disease in Great Britain.

The clinical symptoms and pathological changes associated with trichostrongylosis in grouse and partridges in Great Britain were approximately the

[1] Paper presented at the Ninth Annual Meeting of the American Society of Parasitologists, Boston, Mass., December 28, 1933.

same. Small numbers of the worms apparently did not injure the health; loss of appetite, with resulting emaciation, anaemia and toxaemia were, however, coincidental with heavy infestations. There were thickening and reddening of the caecal walls; small haemorrhages were sometimes present. The caeca ceased to function and their contents decomposed. The largest number of specimens of *T. tenuis* counted from a partridge was 12,226, whereas the smallest number in a partridge showing typical symptoms was 1426; in twenty-one birds, the parasites from which were counted, the average number of worms was 4778. In the grouse there were indications that trichostrongyles enabled bacteria, such as the colon bacillus, to pass through the mucosa of the caeca. This bacterium was always absent from the liver if there were no worms or only a few worms (not exceeding 100) in the caeca; if there were between 100 and 1000 worms, the colon bacillus was sometimes present and sometimes absent from the organs; when more than 1000 trichostrongyles were present, the colon bacillus was always present in the liver and occasionally in other organs.

The first record of *T. tenuis* in the United States was from bob-white quail (*Colinus virginianus*), Cram (1925) reporting it as *T. pergracilis*. Stoddard (1931), who had collected those specimens, subsequently found the parasite in approximately 120 quail in five south-eastern states; the infestations were comparatively light and there was no evidence of any pathological condition associated with them.

Recent epizootics of trichostrongylosis in the United States

With its recent appearance in epizootic form in this country, *T. tenuis* assumes an importance here from a practical as well as a scientific viewpoint. In one outbreak, occurring in the south-eastern part of the District of Columbia, there were heavy losses among young domestic geese 6–8 weeks old. The trichostrongyles were present in large numbers in the caeca, and the mucosa of the caeca was ulcerated and darkly stained with blood pigments. Owing to the fact that renal coccidiosis was also present, the deaths could not be ascribed primarily to the worm infestations. That *T. tenuis* had been present previous to this time in the District of Columbia is indicated by the presence in the collection of the Zoological Division of two hitherto unreported lots of this parasite from the District, one lot collected by W. D. Foster in 1910 from the domestic duck and the other lot collected by one of us (E. B. C.) in 1929 from a blue goose (*Chen caerulescens*), the location in both cases being the caeca. No clinical data are available for the former case; the goose had died at the National Zoological Park, apparently from heavy infestation of the gizzard with species of *Epomidiostomum* and *Amidostomum*.

Another outbreak of trichostrongylosis was brought to our attention from Detroit, Michigan[1]. A flock of pheasants, which had been imported from

[1] We are indebted to Dr Carl H. Shroeder of the Larro Research Farm for furnishing data and material.

England and were being held in captivity, suffered severely from the effects of heavy infestation with *Trichostrongylus tenuis*, whereas pheasants reared on the premises showed no clinical evidence of the parasitism.

Biological observations on *T. tenuis*

Cultures of eggs of *T. tenuis* were made by the authors from caecal droppings or caecal contents of infested pheasants. Two methods were used. One method consisted of smearing the faecal material on glass slides which were laid flat in a Petri dish in a layer of water of a depth just slightly less than the thickness of the slides, and the dish covered; thus an abundance of oxygen and proper moisture was available and, after hatching, most of the larvae crawled to the edge of the slide and over it into the water. The other method consisted of mixing faecal material with a small amount of finely ground animal charcoal into a moderately moist paste which was spread on the bottom of a small Petri dish, the cover of the dish resting directly on the circular rim of the dish; the larvae later ascended the sides of the dish and congregated on the inner surface of the cover in the condensed moisture. The former method had the advantage that the control of moisture content was not so difficult as in the second method, but the latter method was advantageous in that after migration larvae could be secured in purer culture, without faecal contamination.

In order to measure the larvae, they were mounted in water under a coverslip and heat applied for a few moments; it was found that they could be stupefied for a period of 10 min. to half an hour, with subsequent revival, or could be killed with somewhat greater heat, without their total length being affected. In some instances the larvae were stained in order to obtain sharper delineation; neutral red, brilliant cresyl blue or thionin, added to the water containing the larvae before the application of heat, stained the larvae at the moment of death.

Eggs of *T. tenuis* of pheasant origin hatched in 36–48 hours at moderate room temperatures. The youngest larvae, that is, in a 2-day-old culture, had an average length of 380μ (range 350–416μ); in a 4-day-old culture an average of 523μ (range 475–581μ). Larvae migrated as early as the sixth day, at which time they had an average length of 531μ (range 468–634μ) and were infective for the bird host. Up to the fifth day after culturing, the oesophagus of the larva was bulbous, and no cuticular body sheath was evident; the bulb of the oesophagus then became less prominent and in larvae of 6 and 7-day-old cultures the oesophageal bulb had disappeared and a cuticular body sheath was apparent, projecting beyond the tail. No evidence of moulting was seen in the cultures.

Influence of external environmental factors

As regards the length of life of *T. tenuis* in the outside world, under various conditions, the following observations were made. A small proportion of larvae after migration were found to remain alive in a shallow layer of water

for a maximum of 23 days, in two different cultures (periods of January 12th to February 4th, and of September 19th to October 12th) at moderate room temperatures. When drying occurred, the larva coiled and shrank in its sheath; it could be revived by the addition of water after a few days of drying at ordinary room temperatures but was killed by longer periods.

The eggs and larvae showed marked resistance to cold when moisture was present. Eggs in fresh caecal droppings were held at ordinary refrigeration temperature, approximately 7° C. (47° F.), for 5 days, then cultured; 22 hours after culturing, active larvae were present, indicating that a certain amount of development had occurred at the low temperature. These larvae proved infective for two out of three chickens to which they were fed, the one negative result possibly indicating that the virulence had been reduced by the exposure to cold (see discussion below). A period of 35 days at 7° C. (47° F.), on the other hand, resulted in a shrunken appearance of eggs of *T. tenuis* in droppings, and their subsequent inability to develop larvae when returned to a warmer environment; probably a period somewhat shorter than 35 days would have accomplished similar results.

Larvae after migration, however, showed much greater resistance to cold than did the eggs of *T. tenuis*. Ordinary refrigeration temperatures for considerable periods having no apparent bad effects, exposure to more extreme cold was tried. Several dishes, with the larvae in a very small amount of water, were held at an average of about −3° C. (+26·6° F.), the temperature ranging from +3° to −20° C. (+37·4° to −4° F.), and portions removed and examined for viability, at intervals. In the dishes which continued to hold some moisture, larvae remained alive for 4 months, proving infective for a chicken at the end of that period; no longer exposure was tried. On the other hand, where the moisture was so scant that it evaporated and the additional factor of drying was operative, the larvae were all dead at the end of 2 months.

These observations are consistent with those made in England, where it was found that trichostrongyle larvae from grouse and from partridges were not killed by extreme cold but were killed by extreme drought.

Cross-transmission experiments

An attempt to infect two young ducks with *T. tenuis* of goose origin met with no success; one of the ducks was fed a 7-day-old culture and the other duck a 10-day-old culture, without subsequent infestation.

Cultures of the trichostrongyle larvae of pheasant origin were fed to the following birds, with resulting infection or non-infection, as listed:

1 duck, negative; 1 pigeon, negative; 2 turkeys, 1 negative, 1 positive; 1 guinea-fowl, positive; 20 chickens, 2 negative, 18 positive.

The chickens were from 2 weeks to 3 months old at the time of attempted infection; the other birds were adult. One of the cases of failure to infect a chicken involved the use of larvae hatched from eggs which had been held at approximately 7° C. (47° F.) for 5 days (see above); in spite of a second feeding

from the same culture, 13 days after the first feeding, the chick still remained uninfected. The virulence of the culture may, therefore, have been reduced by the cold, in spite of the fact that it infected two other chickens. However, for the other case of non-infection of a chicken, no observations were made which might explain the negative result.

The shortest period in which the parasite developed to maturity, with subsequent passage of eggs in the droppings of the bird, was 7 days.

Size of adult trichostrongyles in different birds

Measurements of adult specimens of *T. tenuis* from different bird hosts showed significant differences, the average total length in mm. being as follows:

Bird	Male	Female
Goose	7·02	7·91
Pheasant	5·50	6·50
Chicken	6·04	7·60
Turkey	Not available	8·70

Since the specimens from the chicken and turkey were developed experimentally from *T. tenuis* originating from the pheasant, the parasites from these three hosts were unquestionably the same strain; the size of the specimens from the goose, possibly a different strain, fall within the size range of the specimens from gallinaceous birds.

Clinical and pathological observations

The principal clinical effect noted in chickens experimentally infected with *T. tenuis* was diarrhoea from the caeca, in early stages of the infestation; mucus and blood were present in the caecal discharge. Later the caeca ceased to function. At necropsy, acute typhlitis was evident; there was thickening of the wall, with engorgement of blood vessels, and the mucosa was covered with a thick layer of blood-stained mucus. Sections of unopened caeca from a chicken, killed 9 days after artificial infection, showed congestion of blood vessels and considerable destruction of the surface of the mucosa in areas where worms were numerous. The contents of the lumen were composed mostly of mucus, with some desquamated epithelium. Most of the trichostrongyles were on or near the surface, but a small proportion had penetrated fully one-half the depth of the mucosa and in a few instances to the basal membrane. Dissolution of the tissues around the embedded worms was apparent. In one instance blood cells were noted in the oesophagus of a parasite. Mitotic division in the epithelial cells was abundantly evident.

There were no deaths from the experimentally induced trichostrongylosis. However, all infestations were fairly light as compared with those in nature which have been the cause of death, and the experiment birds were held under conditions favourable for resisting the disease, as regards temperature, feed and sanitation. The chickens were held in cages with wire-mesh floors, so that re-infection was prevented. In those birds held beyond the acute stages of the infestation, symptoms disappeared after a short period and the caeca gradually

resumed normal function. The infestation tended to die out in about 2 months under such conditions. In several cases live adult worms were found which had been passed in the droppings during the second month of infestation, and in a few chickens which were held beyond the 2-month period, and which proved at necropsy still to have a few trichostrongyles in the caeca, egg production by the parasites had previously ceased or so greatly decreased that it had been impossible to find eggs in the droppings toward the end of the second month.

SUMMARY

Clinical trichostrongylosis, caused by *T. tenuis* in the caeca, previously known in epizootic form among gallinaceous birds in Great Britain, is here reported for the first time in this country, in native domestic geese in the District of Columbia and in imported pheasants in Michigan.

In cultures made from caecal contents of infected pheasants, eggs hatched in 36-38 hours; the infective larval stage might be reached as early as the sixth day (fourth day after hatching). Moisture being present, migrating larvae, retracted within the cuticular sheath, resisted an average temperature of about −3° C. (range +3° to −20° C.) for 4 months, proving infective for a bird host at the end of that period. The chicken, turkey and guinea-fowl were successfully infected with cultures derived from pheasants; attempts to infect the duck and pigeon with cultures from pheasants, and to infect the duck with cultures from geese, were unsuccessful. Development to adults in bird hosts took place in a minimum of 7 days after infection; the size of adults varied in different birds, being greater in the chicken than in the pheasant, and still greater in the turkey. In early stages of infection diarrhoea from the caeca, with passage of mucus and blood, occurred; later all discharge from the caeca ceased. The pathological picture was that of acute typhlitis, with the trichostrongyles lying in close contact with or penetrating the mucosa. Reinfection being prevented, symptoms were of fairly short duration, and the infestation tended to die out in about 2 months.

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[1] For other references, see previous paper by Cram and Wehr, p. 339.

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A NEW SPECIES OF ACCACOELIID TREMATODE (*ACCACLADOCOELIUM ALVEOLATUM* N.SP.) FROM THE INTESTINE OF A SUN-FISH (*ORTHAGORISCUS MOLA* BLOCH)

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(With Plate XIII)

The following is a description of a new species of Accacoeliid Trematode found in the intestine of a sun-fish (*Orthagoriscus mola* Bloch), caught by a fisherman in June, 1933, at Salcombe, Devon. The fish was examined by Mr J. W. Poulton of Downing College, Cambridge, to whom my thanks are due for bringing me the parasites for identification. In addition to the species described in this paper the host contained the following forms: on the skin, *Tristoma molae* and *Lepeophtheirus nordmanni*; in the intestine, *Ancistrocephalus microcephalus*.

General appearance (Fig. 2)

The worms measure on the average 6·5 mm. in length and 1·5 mm. in maximum breadth. In these specimens, which had been killed and preserved in formol, the body is rather stout and does not taper very much towards either end. It is curved ventrally and circular in cross-section. The cuticle is much wrinkled, especially about the base of the ventral sucker. This organ therefore is very possibly pedunculated in life, and the whole animal is probably quite slender and capable of great extension and retraction. In correlation with this the body wall is thick and the muscular layers well developed. The two suckers lie at the anterior end of the body, about 1·0 mm. apart. The ventral sucker is 1·0 mm. across, the oral sucker 0·5 mm.

At first sight the worm appears to have a third sucker at its posterior end, but this appearance is due to the slight in-tucking of the part of the body bearing the wide excretory pore and it is accentuated by the presence in this region of a zone of close-set skin "papillae," which deepen the invagination. This zone is about 0·25 mm. wide and extends completely round the body.

The "papillae" are very peculiar structures (Figs. 4, 5). In sections they take up the stain rather strongly and present a finely granular appearance (*pa*). They contain no nuclei and lie entirely outside the cuticle. They are roughly spindle-shaped and are ragged at their free ends. They are closely crowded together and under an oil-immersion lens it is evident that the spaces between them are occupied by very thin layers of cuticle. In fact, serial sections show that the cuticle in this region is raised into a large number of

ridges or partitions, which cross one another almost at right angles. The cuticle thus resembles a honeycomb, with "cells" about 0·08 mm. deep. The free edges of the partitions are in some places swollen into flanges, in which the cuticle exhibits a vesicular structure (Fig. 5, *ve*). Without access to living material it is impossible to determine the nature of the spindle-shaped "papillae." The absence of nuclei indicates that they may be simply small masses of débris from the intestine of the host, which have been trapped in the honeycomb spaces, or that they may consist of some kind of secretion. The latter hypothesis is probably not correct, since no gland cells were observed in the neighbouring tissues, and the most careful examination failed to reveal any connection between the "papillae" and the subcuticular structures.

Alimentary canal (Fig. 2)

The mouth opens on the ventral edge of the oral sucker. It leads into a poorly developed pharynx, from which the narrow oesophagus runs backwards for a short distance and then turns sharply in a dorsal direction. The oesophagus divides at the level of the anterior margin of the ventral sucker into right and left branches, which come off almost at right angles. From the point where these two transverse limbs arise four small diverticula are given off, which run forward for a short distance and then end blindly. Similar structures have been recorded in other Accacoeliidae (Looss, 1912; Odhner, 1927 and 1928). From the right and left limbs the anterior and posterior intestinal caeca run forwards and backwards. The whole intestine is thus H-shaped, as is typical of the family. As they run down the length of the body the caeca give off a number of blind diverticula, which are very voluminous and usually filled with brownish food material. They are very conspicuous features in a whole mount and push their way among the other organs. Each of the anterior caeca has six of these diverticula, while those borne by the posterior caeca are more numerous and larger.

The posterior caeca do not end blindly, but communicate by wide pores with the excretory bladder—a feature which Odhner (1928) has found to be common to the members of the Accacoeliidae. The specimens I have examined were considerably contracted, and it is possible that during life the so-called excretory bladder is really part of the outer surface of the animal, or at least is capable of being evaginated. Many of the closely allied Hemiuridae are able to tuck in or protrude the hind end of the body; and further the lining of the bladder in the species under discussion is indistinguishable microscopically from the general cuticle covering the body (Fig. 3). This cuticle ceases abruptly at the sites of entry of the two "ani" into the bladder.

Excretory system

The excretory tubes are Y-shaped, the stalk of the Y entering the bladder between the two ani. From the limbs of the Y a series of tubes ramifies all over the body.

Genital system (Fig. 1)

The genital pore is situated in the mid-ventral line immediately behind the mouth. There is a narrow genital atrium, or, to use Manter's term (1926), a "sinus sac," with a cuticular lining, and the walls of this sac closely invest the genital papilla which projects from its dorsal wall. The papilla is about 0·1 mm. long in the specimens examined and it is doubtless capable of protrusion from the sinus sac, since it is provided with muscle fibres. It contains the hermaphrodite duct or genital sinus, formed by the fusion of the male and female ducts.

Male organs. The anterior testis lies just behind the middle of the ventral sucker and is slightly more dorsal than the posterior testis, which is immediately behind it. The two testes are thus in what Odhner (1928) calls the "*Orophocotyle* position."

From its opening into the genital sinus the male duct runs dorsally and backwards, following a rather winding course through the centre of the body. Inside the genital papilla its calibre is very minute and it is invested by muscle fibres, but it soon becomes surrounded by an enormous prostate gland. After leaving the prostate the male duct turns backwards and becomes the seminal vesicle. This is a long coiled tube with wide calibre and very thin walls. At the front end of the anterior testis the seminal vesicle receives the two vasa deferentia. One vas deferens is short and issues from the dorsal surface of the anterior testis; the other is longer and comes from the left side of the posterior testis.

Female organs. The ovary lies immediately behind the posterior testis and is provided with a well-marked sheath. This sheath is distinct from that investing the shell gland, which lies near its right side.

In the genital papilla the metraterm is ventral to the male duct and it passes backwards, becoming rapidly wider. It runs ventral to the prostate, which is partially wrapped round it. About the middle of the ventral sucker the metraterm becomes the uterus. There are two sets of uterine coils. Those which form the continuation of the metraterm are confined to the dorsal half of the body and they extend to the posterior end of the worm. Here the uterus turns forwards again and its coils can be traced along the ventral half of the body until they reach a point just below the testes. Here the uterus turns backwards again, and, narrowing, becomes the oviduct. The intestinal caeca with their diverticula lie between the dorsal and ventral sets of uterine coils.

The oviduct is a thin-walled coiled tube which enters the right side of the ovary between this organ and the shell gland. It gives off Laurer's canal, which runs upwards and opens on the dorsal surface above the ovary.

The arrangement of the vitelline apparatus presents an interesting peculiarity. The main vitellarium is situated entirely in the anterior region above the oral sucker. It does not extend further back than the posterior margin of the ventral sucker. In a cleared specimen, mounted whole, the yolk tissue has the

appearance of a dark hood or cap on the head end. A single vitelline duct runs backwards, dorsal to the testes. Just behind the shell gland it unites with another duct to form the common vitelline duct, which immediately enters the oviduct. This second vitelline duct is short and stout and it drains a single large vitelline follicle lying on the right side of the body just behind the level of the ovary. Thus one vitellarium is large and confined to the front end of the animal, while the other is reduced to a single follicle and lies in the posterior part of the body. This state of affairs recalls the description given by Looss of the vitelline arrangements in the Accacoeliid worm, *Tetrochetus raynerius*. Here there is one main vitellarium, which lies near the ventral sucker and is drained by a single duct. This is joined by another shorter duct draining only six or seven follicles, situated near the ovary. A few of the Accacoeliidae have gone further and completely lost one vitellarium and duct, possibly in correlation with a slender body form (*Accacladium serpentulus*, *Orophocotyle planci* and *O. divergens*).

Systematic position

The presence on the intestine of large, pouch-like diverticula, six of which spring from each anterior caecum, and the structure of the genital papilla show that the worm must be placed in the genus *Accacladocoelium* (Odhner, 1928). It differs, however, from the three known species of this genus in the following features:

(1) The peculiarities in the vitelline apparatus described above.

(2) The "honeycomb" at the hind end of the body.

The last-named structure being a prominent feature, the name *Accacladocoelium alveolatum* n.sp. is proposed for this worm.

Finally, since the literature relating to the Accacoeliidae is somewhat scattered, a list of the members of the family, with their hosts, is appended. They are all intestinal parasites:

In *Orthagoriscus mola*:

Accacladocoelium nigroflavum Rud. 1819.
A. macrocotyle Dies. 1858.
A. petasiporum Odhner 1928.
A. alveolatum n.sp.
Accacoelium contortum Rud. 1819.
Accacladium serpentulus Odhner 1928.
Orophocotyle foliatum Linton 1898.
Rhynchopharynx paradoxa Odhner 1928.

In *Ranzania truncata*:

Orophocotyle planci Stossich 1899.
O. divergens Looss 1902.

In *Ausonia cuvieri*:

Tetrochetus raynerius Looss 1912.

Diagnosis. Characters of the genus *Accacladocoelium.* Length 6·5 mm., maximum breadth 1·5 mm. Diameter of oral sucker 0·5 mm., of ventral sucker 1·0 mm. Ventral sucker prominent, about 1·0 mm. behind oral sucker. Encircling the body immediately in front of the wide excretory pore there is a zone about 0·25 mm. broad, which bears a peculiar cuticular structure, resembling a honeycomb, the septa of which are about 0·08 mm. deep. The main vitelline mass is confined to the region anterior to the ventral sucker, but there is one vitelline follicle at the level of the ovary; from this a short tube joins the main vitelline duct.

Eight specimens of this worm were taken from the sun-fish. One of these is in the possession of the collector, Mr Poulton, and two complete specimens are in the collection of the Molteno Institute (No. 773). In addition one specimen has been deposited in the Museum of Comparative Anatomy, Cambridge.

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EXPLANATION OF PLATE XIII

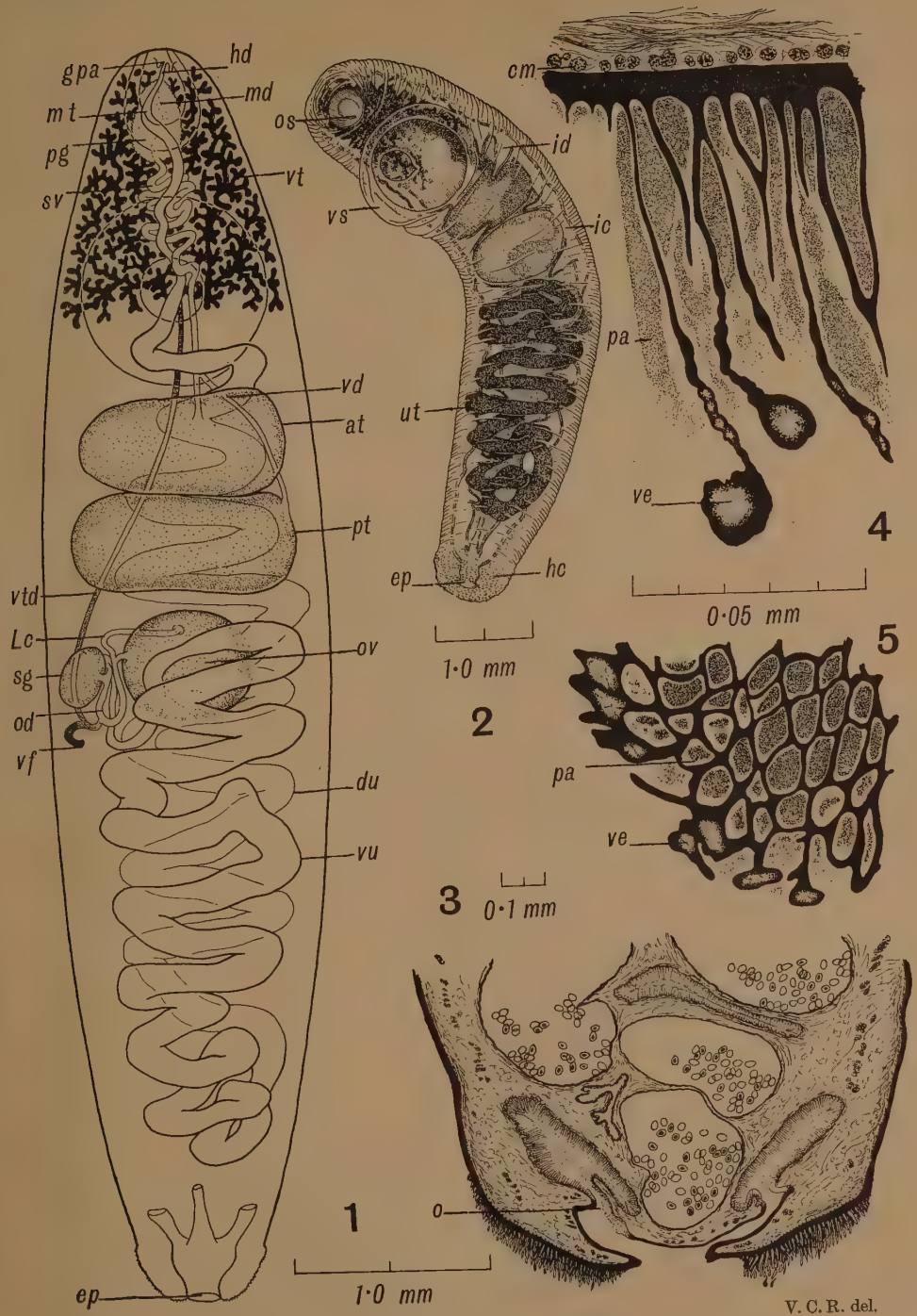
Fig. 1. Semi-diagrammatic reconstruction of genital system. The coils of the oviduct have been simplified.

Fig. 2. Camera drawing of cleared specimen. The dark contents of the intestinal caeca have been omitted for the sake of clarity.

Fig. 3. Longitudinal section through hind end, to show the "honeycomb" and the two posterior intestinal caeca opening into the excretory bladder; cuticle in black.

Fig. 4. Longitudinal section through "honeycomb"; cuticle in black.

Fig. 5. Tangential section through "honeycomb"; cuticle in black.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Lettering

at, anterior testis.
cm, circular muscles.
du, dorsal coils of uterus.
ep, excretory pore.
gpa, genital papilla.
hc, honeycomb.
hd, hermaphrodite duct.
ic, intestinal caeca.
id, intestinal diverticula.
Lc, Laurer's canal.
md, male duct.
mt, metraterm.
o, opening of intestinal caecum into excretory bladder.
od, oviduct.
os, oral sucker.
ov, ovary.
pa, "papillae."
pg, prostate gland.
pt, posterior testis.
sg, shell gland.
sv, seminal vesicle.
ut, uterus.
vd, vasa deferentia.
ve, vesiculation in septa of honeycomb.
vf, posterior vitelline follicle.
vs, ventral sucker.
vt, vitellarium.
vtd, vitelline duct.
vu, ventral coils of uterus.

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A REVISION OF THE GENUS *RHADINORHYNCHUS* (ACANTHOCEPHALA) WITH DESCRIPTIONS OF NEW GENERA AND SPECIES

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(With Plate XIV)

The genus *Rhadinorhynchus* was erected by Lühe (1911) with *R. pristis* (Rud. 1802) as the type and only species. Since that time a considerable number of other species have been assigned to this genus, and a summary of these is given in Table I. It will be observed from this table that it is proposed to place these

Table I

Species	Original description	Assigned to *Rhadinorhynchus*	Here referred to
pristis	*Echinorhynchus pristis* Rudolphi 1802	Lühe, 1911	*Rhadinorhynchus*
horridus	*Rhadinorhynchus horridus* Lühe 1912	Lühe, 1912	*Rhadinorhynchus*
ornatus	*Echinorhynchus pristis* Linton 1892	Van Cleave, 1918	*Rhadinorhynchus*?
tenuicornis	*Echinorhynchus pristis* var. *tenuicornis* Linton 1892	Van Cleave, 1918	*Rhadinorhynchus*
medius	*Echinorhynchus medius* Linton 1908	Van Cleave, 1918	*Gorgorhynchus*
selkirki	*Rhadinorhynchus selkirki* Van Cleave 1920	Van Cleave, 1920	*Rhadinorhynchus*
exilis	*Rhadinorhynchus exilis* Van Cleave 1928	Van Cleave, 1928	*Rhadinorhynchus*
katsuwonis	*Rhadinorhynchus katsuwonis* Harada 1928	Harada, 1928	*Nipporhynchus*
wheeleri	*Rhadinorhynchus wheeleri* Baylis 1929	Baylis, 1929	*Aspersentis*
johni	*Rhadinorhynchus johni* Baylis 1929	Baylis, 1929	*Aspersentis*

species in at least four different genera. The species *horridus*, *tenuicornis*, *exilis* and *selkirki* undoubtedly belong with the type species *pristis*, in the genus *Rhadinorhynchus*. As can be seen from the summary of their characters in Table II, all of these except *selkirki* seem to be valid species with well-defined characters, but *selkirki* appears to be identical with *pristis*, and the name is herein considered a synonym of *pristis*. The species *wheeleri* and *johni* are unquestionably congeneric with *Aspersentis austrinus* Van Cleave 1929, and belong in the genus *Aspersentis* if this is accepted as a valid genus, which in my opinion it should be, on the basis of the short proboscis with very widely different dorsal and ventral hooks, the short lemnisci, and the pyriform cement

Table II. *Summary of characters of species of* Rhadinorhynchus.

Species	Size mm.	Proboscis hooks: No.	Proboscis hooks: Small basal hooks longitudinally arranged	Proboscis hooks: Special large basal row	Body spines	Cement glands	Testes	Remarks
pristis	18–76 × 0·56–1·12	14 × 26	None	A ring, not separated by a space	Few scattered ones in rings, then unspined space, then scattered ones on ventral side to beyond end of proboscis sac, dorsal side practically free	No.? "schlauchformig." Nearly as long as two testes together	Contiguous 1·7 × 0·35–0·4 mm.	Type
ornatus	12–13 × 0·8	24 × 40	None	A ring, not separated by a space	Sparsely scattered	Males unknown	—	Possibly belongs with *katsuwonis* in *Nipporhynchus* n.gen.
tenuicornis	4·5–16 × 0·3–0·56	12–14 × 22–24	Present	A crescent separated by a space	Anteriorly in rings, then scattered, extending to middle of proboscis sac on ventral side	Eight, tubular, with club-like enlargements proximally	Contiguous 0·25 × 0·13 mm.	—
horridus	19 × 0·75	14–16 × 31	None	A ring, not separated by a space	Anteriorly in rings, followed by scattered ones of enormous size	Male unknown	—	—
selkirki	6–20 × 0·46–0·8	12–14 × 24	None	A ring, not separated by a space	As in *pristis*	Not mentioned	Not mentioned	Apparently identical with *pristis*
exilis	12 × 0·96	12 × 32	None	None	Minute, inconspicuous	Male unknown	—	Peculiar in lacking basal row of spines on proboscis

glands. The species *tenuicornis* was first described as a variety of *pristis* by Linton (1892). Additional information concerning its morphology was given by the same writer in 1905 (p. 380), and Van Cleave contributed still more in 1918, yet no complete description of the worm is available. Linton gives a brief but inadequate description of the internal organs, while Van Cleave omits this entirely; neither writer accurately describes the spiny armature of proboscis and body. Since this is the situation a new specific description of this species is given below.

Rhadinorhynchus tenuicornis Van Cleave 1918

Specific diagnosis. Body slender, but enlarged at about the level of posterior end of proboscis sac and sometimes markedly inflated. Proboscis usually bent at angle to body. Length of males 4·5–6 mm. (according to Linton 7·4 -10·2 mm.); of females 7–10 mm. (according to Linton 10–16 mm.). Maximum diameter of males 275–300μ. Females about 250–300μ in diameter in cylindrical posterior portion, expanding to 350–550μ anteriorly. Proboscis 1–1·3 mm. long, slightly broader anteriorly than at middle or base; maximum diameter 65–90μ. Proboscis armed with 12–14 longitudinal rows of hooks with about 22–24 hooks in each row. Anterior 16–18 of these hooks arranged in the usual spirals, but about six hooks at the posterior end of each row do not show an obvious spiral arrangement but appear to lie in vertical rows. Hooks in anterior spirals fairly uniform in length, ranging from about 30–40μ, but those on dorsal side much slenderer and less curved than those on ventral side, and with less well-developed roots. Dorsal hooks about 5μ in diameter, ventral ones about twice this diameter and curved through 90°, having somewhat the shape of grappling hooks. Hooks in vertical rows at base of proboscis very small and close behind each other, most of them broad, strongly curved, and about 10–15μ long, but a few on dorsal side slender and less curved. Shortly behind last of these small hooks a ventral crescent of eight large, slightly curved hooks 40–50μ in length, projecting almost at right angles to proboscis. Proximal fourth of proboscis bare. Anterior part of body armed with characteristic, large, sheathed spines in rather irregular arrangement, although there are two or three fairly regular rows of large spines, 60–75μ long, just behind junction with proboscis; behind these anterior rows spines become more and more scattered and smaller in size. Ventral spines somewhat larger than dorsal ones, and extending somewhat farther posteriorly, to behind middle of length of proboscis sac. Proboscis sac somewhat longer than proboscis. Lemnisci tubular, with slender finger-like endings, about one and one-third times the length of proboscis sac. Male with well-developed copulatory bursa, about as wide as long. Testes posterior to middle of body, anterior border of anterior testis about 1·5–2 mm. from posterior end. Testes one behind the other, contiguous, each about 250μ long and half as wide. Sperm ducts large, with bulging lobe-like diverticula, uniting into a single vas deferens which dilates into a bulb-like seminal vesicle posteriorly. Cement glands eight in number, tubular with club-like enlargements containing

groups of five or six nuclei, just posterior to the testes. A large sac-like cement reservoir present. Eggs with thin outer membrane, and thick inner envelope drawn out into knob-like processes at each end. Eggs about 80μ long and 12–18μ broad, the embryo inside about 55–60μ long.

This species has been recorded by Linton from a large number of species of marine fishes, but much more commonly at Beaufort, N.C., than at Woods Hole. My specimens have been obtained only from *Micropogon undulatus* and *Leiostomus xanthurus*, both Sciaenid fishes. In both these fishes it is a common parasite in Galveston Bay.

The species *katsuwonis* is certainly not congeneric with *tenuicornis*, for the latter has eight pyriform cement glands, whereas *katsuwonis* has only four very elongated tubular cement glands. The number of cement glands in other species of *Rhadinorhynchus* has not been mentioned, but it is probable that if the type species *pristis* had less than the usual eight, the fact would have been mentioned by Lühe. The similarity in other respects between *pristis* and *tenuicornis* makes it fairly safe to assume that the latter is a true *Rhadinorhynchus*. *Katsuwonis*, therefore, must be removed to a new genus, for which the name *Nipporhynchus* is proposed. The following provisional generic characterisation is based on Harada's description:

Nipporhynchus n.gen. Proboscis armed with over twenty longitudinal rows of hooks, with thirty-two or more hooks in each row; ventral hooks stronger and more curved than dorsal. A circle of prominent arcuate hooks at base of proboscis. Anterior region of body with scattered spines. Proboscis sac cylindrical, double-walled, much longer than proboscis. Lemnisci ribbon-shaped, shorter than proboscis sac. Testes elongate, one behind the other. Cement glands four in number, long, cylindrical, one-fourth or more length of body, and entirely filling body cavity posterior to testes. Eggs elongate with outpocketings of middle egg membrane at poles.

In the character of the proboscis, and in other features as far as they were given, Linton's species *ornatus* resembles *katsuwonis*. Unfortunately, however, no mention is made of the cement glands or of the lemnisci. For the present there is no sound reason for removing *ornatus* from the genus *Rhadinorhynchus* to *Nipporhynchus*, but it is possible that such a change will be necessary when more information concerning Linton's species becomes available.

The species which Linton (1908) described as *Echinorhynchus medius*, and which Van Cleave transferred to the genus *Rhadinorhynchus* in 1918, resembles a new species, described below, so closely that there might even be some question of their specific distinctness. A study of this new species makes it clear that in describing *medius* Linton made an error in mistaking the extremely slender and elongate cement glands for the vas deferens, while the structure which Linton interpreted as a cement gland is in reality either a cement reservoir or seminal vesicle opening independently into the ejaculatory chamber. Van Cleave (1918) re-examined Linton's type material but apparently failed to detect these misinterpretations. A new genus, for which the name

Gorgorhynchus is proposed, is therefore erected to contain the new species, *Gorgorhynchus gibber*, as type, and Linton's *medius*. The genus *Gorgorhynchus* is characterised as follows:

Generic diagnosis. Proboscis cylindrical or fusiform, stout, densely armed with numerous rows of strong recurved hooks, not appreciably different in size or form on ventral and dorsal sides. Neck smooth, conical. Anterior part of body armed with irregularly arranged spines ensheathed in cuticular folds, sometimes more extensive ventrally than dorsally. Body slender, expanded and bent ventrally in the anterior third. Proboscis sac about twice as long as proboscis. Lemnisci long and slender. Testes situated in anterior half of body, not contiguous. Cement glands four in number, very long and slender. Type species, *Gorgorhynchus gibber*.

The conspicuous points which distinguish this genus from *Rhadinorhynchus* are the shape and armature of the proboscis, the absence of any appreciable difference between dorsal and ventral proboscis hooks, more densely armed and more sharply defined spiny area on the body, the anterior position of the well-separated and nearly round testes, and particularly the very elongate and slender cement glands which are only four in number. It is possible that *Nipporhynchus* represents a transition between *Rhadinorhynchus* and *Gorgorhynchus*, since it too has the cement glands reduced to four, and the proboscis larger and more heavily armed, but in it the cement glands occupy nearly the entire body cavity behind the testes. Both in general form and armature of the proboscis and in the form of the cement glands, *Gorgorhynchus* has a marked resemblance to *Arhythmorhynchus* and *Centrorhynchus*.

Following is a description of the new species which is made the type of the genus:

Gorgorhynchus gibber n.sp.

Specific diagnosis. Body elongated and slender, expanded in its anterior fourth, and bent ventrally so that proboscis lies at an angle of about 45° with rest of the body. Greatest diameter behind end of proboscis sheath. Males and immature females from 13 to 21 mm. long, with a diameter of about 0·6 mm. in the posterior two-thirds of the body, widening out to about double this diameter in the expanded region anteriorly. Posterior portion of body elongate and bluntly rounded at end. Proboscis 2½–3 times as long as wide, the dorsal contour approximately straight, the ventral contour slightly convex; length about 1·25 mm., diameter 0·4–0·5 mm. Hooks arranged in about twenty-four longitudinal rows of eighteen hooks each. Hooks at anterior end of proboscis large and powerful, 75–80μ long, with a blade 16μ broad, gradually changing behind middle of length of proboscis to very strongly curved hooks with out-curved tips, resembling grappling hooks, measuring about 65μ from tip to farthest point of curve, and with blade about 24μ broad; these again change in the basal three or four rows to slender hooks only slightly curved, 70–75μ long and 10μ broad. The proboscis is followed by an unarmed neck measuring about

0·45 mm. long and about 0·4 mm. broad where it joins the proboscis, and 0·6 mm. broad where it joins the body. Body armed anteriorly with about 11–13 irregular rows of cuticle-covered spines, 60–70μ long, occupying about 0·75–0·85 mm. of the anterior part of the body, thus extending somewhat beyond middle of length of proboscis sac, but only slightly farther on ventral than on dorsal side. Proboscis sac about 2·2 mm. long and 0·6 mm. in maximum diameter. Lemnisci broad and flat near end of proboscis sac, then becoming finger-like, and terminating just behind swollen portion of body, at about junction of anterior and middle thirds of total length, in males at about level of anterior testis. Testes 0·3–0·5 mm. in diameter, nearly round or slightly oval, separated from each other by about diameter of a testis, sometimes more, sometimes less. A prominent long-oval vesicle, either a cement reservoir or seminal vesicle, opening independently into ejaculatory chamber. Cement glands extremely long and narrow, 6–7 mm. long in specimens measuring 15–16 mm. in total length, all four so closely associated as to appear like a single duct, with a diameter of only 45–90μ.

This species was found in two out of three specimens of the marine catfish, *Galeichthys felis*, taken near Bolivar Point in the Gulf of Mexico. It resembles Linton's (1908) species *medius* very closely in its general features, but differs in structural details as shown in the following table:

	G. medius	*G. gibber*
Length	♂ 42 mm. ♀ 54 mm.	♂ 16–21 mm. ♀ (im.) 20 mm.
Diameter	♂ anterior 0·75 mm. maximum 1·35 mm. middle 1·00 mm.	♂ anterior 0·6 mm. maximum 1·2 mm. middle 0·6 mm.
Proboscis	1·4 × 0·45 mm.	1·25 × 0·5 mm.
Proboscis sheath	3 × 0·67 mm.	2·2 × 0·6 mm.
Base of neck to first testis	12 mm.	4 mm.
First to second testis	6 mm.	0·5 mm.
Lemnisci extend beyond end of sheath	5·4 mm.	3 mm.
Length of proboscis hooks	Near base 45μ, others 60μ	Near base 70μ, others 70–80μ
Number of hooks	22 vertical rows, 20 hooks each	24 vertical rows, 18 hooks each
Size of body spines	30–45μ long	60–70μ long
Area of body spines	Extend $\frac{1}{3}$ length of sheath on concave side, half as far on convex side	Extend $\frac{1}{2}$ or more length of sheath on each side

The most striking points of difference are the greater size of the hooks on a smaller worm, the distance between the testes, and the distribution of the body spines. In Linton's description, the male genitalia are described as consisting of a pair of testes, a vas deferens, and a long oval cement gland near the posterior end. This, as noted above, is obviously an error, since the structure which Linton took to be a vas deferens is in reality the group of four closely approximated and very elongate cement glands, while the structure described by Linton as a cement gland is in reality either a seminal vesicle or a cement reservoir.

SUMMARY

Of the ten species which have been referred to the genus *Rhadinorhynchus* four (*pristis*, *horridus*, *tenuicornis* and *exilis*) are considered valid members of that genus. *Selkirki* is considered a synonym of *pristis*. *Katsuwonis* is placed in a new genus, *Nipporhynchus*, and it is suggested that *ornatus* may possibly belong with it. *Medius* is transferred to the new genus *Gorgorhynchus* of which a new species, *G. gibber*, is made the type. *Wheeleri* and *johni* are both referred to the genus *Aspersentis*.

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EXPLANATION OF PLATE XIV

Fig. 1. Anterior end of body of *Rhadinorhynchus tenuicornis*, showing spines of proboscis and body, proboscis sac, and lemnisci.

Fig. 2. Posterior end of body of male *Rhadinorhynchus tenuicornis*, showing testes, sperm ducts with lobe-like enlargements, seminal vesicle, cement glands, cement reservoirs, and copulatory bursa.

Fig. 3. Spines of *Rhadinorhynchus tenuicornis*: *a*, body spine, side view; *b*, body spine, front view; *c*, small spine from near base of proboscis; *d*, spine from crescent at base of proboscis; *e*, spine from anterior end of proboscis.

Fig. 4. Egg of *Rhadinorhynchus tenuicornis*.

Fig. 5. *Gorgorhynchus gibber*, male.

Fig. 6. Anterior end of body of *Gorgorhynchus gibber*, showing spines of proboscis and body.

Fig. 7. Spines of *Gorgorhynchus gibber*: *a*, from near anterior end of proboscis; *b*, from a little behind middle of proboscis; *c*, from near base of proboscis; *d*, from body.

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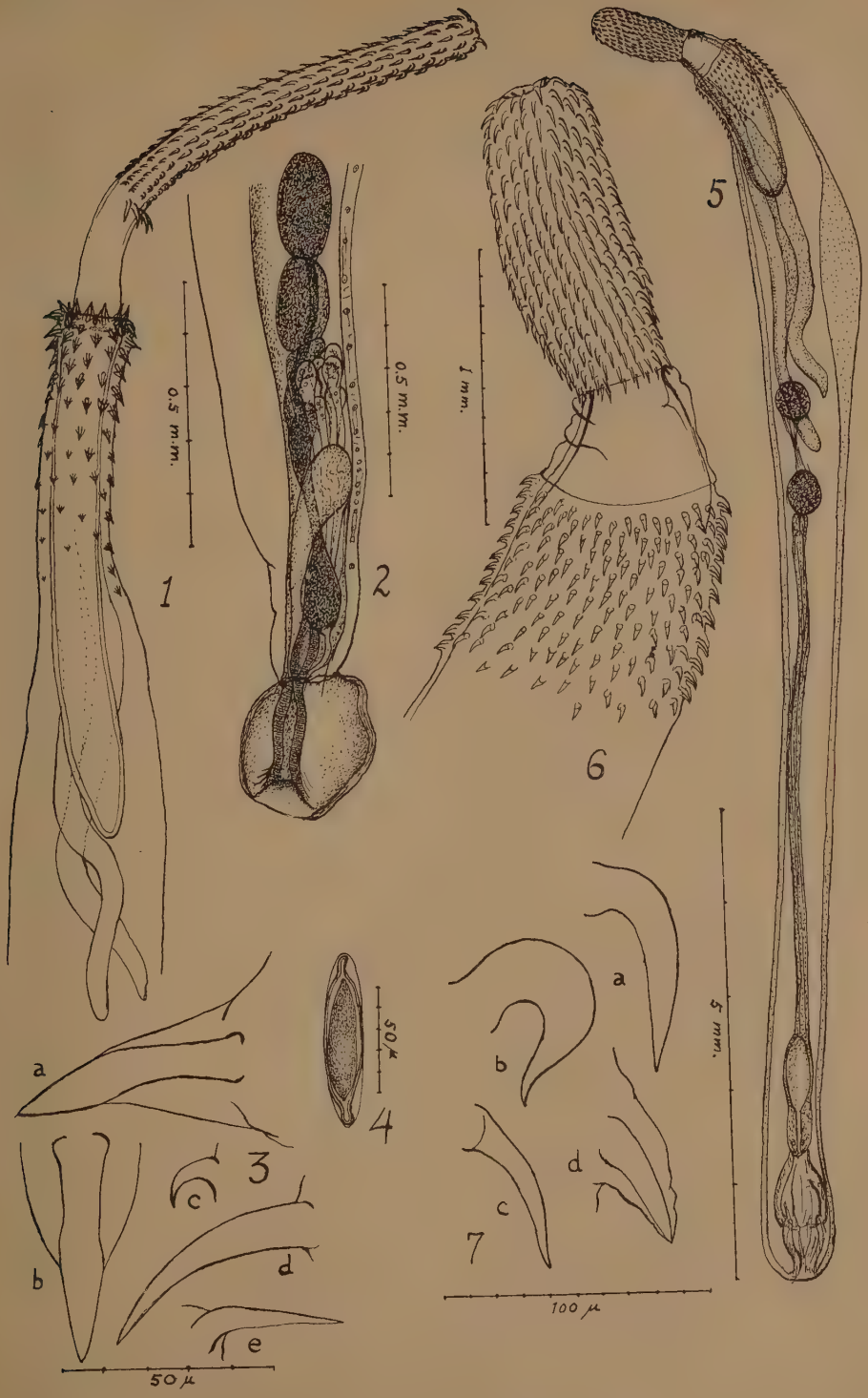
1
0.5 m.m.
2
0.5 m.m.
1 m.m.
5
6
5 m.m.
a
b
c
d
e
3
50 μ
4
50 μ
a
b
c
d
7
100 μ

FIMBRIARIA FASCIOLARIS IN THE PROVENTRICULUS OF A SWAN ASSOCIATED WITH BACTERIAL INFECTION AND ULCER FORMATION

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The adult stages of cestode worms are well known to be very restricted as regards their situation in the host, being almost invariably confined to the small intestine. *Stilesia hepatica* is the most notable exception to this rule, and one or two others have occasionally been observed: Joyeux (1927) mentions *Atriotaenia parva* and *Hepatotaenia festiva* as found in the bile ducts, and *Thysanosoma actinoides* and *Hymenolepis microstoma* as occasionally invading the bile ducts, though normally found in the small intestine. Baer (1926) records three abnormal occurrences of adult Cestodes in the abdominal cavity of birds; these occurrences presumably being accidental, as in each instance the species involved was normally a parasite of the small intestine, and Southwell (1930) mentions *Nematotaenia dispar* as having been found in the pericardial sac of a frog.

To my knowledge *Fimbriaria fasciolaris* has not previously been reported as occurring anywhere other than in the small intestine of the avian host, and the finding of a heavy infestation in the proventriculus is therefore of interest.

The swan in which the worms were found was one of a number which had died of some epidemic disease. At the time of making the post-mortem examination six swans had died, twelve were suffering from severe paralysis, diarrhoea and great weakness, others showed milder symptoms of illness. The small intestine of the specimen which was sent to the laboratory was heavily infested with the acanthocephalid, *Polymorphus boschardis*; no other parasites were found in this part, but on examining the proventriculus a tangled bunch of the strobila of what must have represented some thirty or forty specimens of *Fimbriaria fasciolaris* was recovered. The material had begun to decompose before it arrived at the laboratory and most of the tapeworms had broken away from their attachment to the proventriculus, but careful examination showed some portions still to be adhering to the wall, close to its junction with the gizzard. The mucous membrane in this region was deeply ulcerated and it seemed that the occurrence of *F. fasciolaris* in this unusual site had been responsible for the fatal disease.

On examining sections of the wall of the proventriculus, the heads of several of the worms were observed, some deeply buried in the mucous membrane, and the extensive nature of the ulceration was revealed. Large colonies of bacteria,

showing bipolar staining, were however also observed, and it is possible that this organism and not the Cestode was the direct cause of the lesions and of the disease.

SUMMARY

The unusual occurrence of an infestation of *Fimbriaria fasciolaris* in the proventriculus of a swan is recorded.

The worms were associated with the presence of bipolar staining bacteria and ulcer formation near the site of their attachment.

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NOTES ON MITES COLLECTED FROM THE ISLE OF LEWIS, OUTER HEBRIDES

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I. Introduction

A collection of mites from the Isle of Lewis was sent to me for determination by Mr C. Elton of the Bureau of Animal Population, University Museum, Oxford. These mites were among the parasites collected from the two kinds of mice that occur in Lewis: *Apodemus hebridensis* (de Winton)—the Hebridean field mouse—and *Mus musculus* (Linnaeus)—the house mouse. Mr Elton has published (1934) a separate account of the Nematode and insect parasites found on these two species of mice in Lewis.

The collection consisted of about 500 mites belonging to the families Ixodidae, Gamasidae, Laelaptidae, Acaridae, Tyroglyphidae, Haemogamasidae, Cheyletidae, Ascaidae and Oribatidae. Many of these were collected from the mice themselves, but by far the greater number were obtained from samples taken from the nests of *Mus musculus* with the aid of a Berlese funnel. In the following list, the mites are divided into three groups:

(A) *True parasites*, *i.e.* those mites which suck the blood of the host or burrow into its skin.

(B) *Casual inhabitants*, *i.e.* those mites whose exact relationship with the host is unknown and which are also found free-living. These mites probably act as scavengers.

(C) *Accidental visitors*, *i.e.* mites which have wandered into the nest accidentally or have become entangled in the fur of the mouse.

Two tables are also included in this paper showing the relative numbers of mites that were found on the mice and in their nests respectively.

II. List of mites

(A) *True parasites*

Family Ixodidae. One female and one larva of *Ixodes tenuirostris* (Neumann) were taken from the fur of *Mus musculus*. This species is commonly found on small rodents and frequently occurs in large numbers when in the larval stage.

Family Acaridae. A large number of specimens of a species of *Notoedres* were found on *Apodemus*. The mites formed small scaly patches on both pairs of legs and appeared to be similar to those found infesting *Apodemus sylvaticus* in England (Elton, Ford and Baker, 1931).

Table I. *Mites from mice, Isle of Lewis, collected by C. Elton, August,* 1933.

Ref. No. of tube ...	1	13	17	18	19	26	27	28	29	35	36	47	48	2	3	8	10	22	30	31	32	42
Total No. of mites ...	1	1	Nu	1	1	2	1	1	2	1	1	3	10	2	Nu	Nu	1	1	1	6	12	33
Host ...	M	M	M	M	M	M	M	M	M	M	M	M	M	A	A	A	A	A	A	A	A	A
Locality ...	B	S	S	S	S	S	S	S	S	S	S	S	S	B	S	B	S	S	S	S	S	S
Species of mite:																						
Eulaelaps stabularis	—	—	—	—	—	1♀	—	—	—	1♀	—	—	—	2♀	—	—	—	—	—	—	5♀	—
Laelaps festinus	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1♀	4♀	—	21♀, 1 N_2, 11♂
Ichoronyssus carnifex	—	—	—	—	—	1♀	—	—	—	—	—	1♂, 2♀	8♀	—	—	—	—	—	—	1♀	7♀	—
Gamasus sp.	—	1 N_2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1 N_2	—	—
Parasoides carabi	1 N_2	—	—	—	—	—	—	—	—	—	1 N_2	—	—	—	—	—	—	—	—	—	—	—
Haemogamasus michaeli	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1♀	—	—	—	—
Ixodes tenuirostris	—	—	—	1♀	1 L	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Notoedres sp.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Nu	Nu	—	—	—	—	—	—
Asca affinis	—	—	—	—	—	—	—	1 N_2	1 N_2	—	—	—	2 N_2	—	—	—	1 N_2	—	—	—	—	—
Myobia musculi	—	—	Nu	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cosmolaelaps claviger	—	—	—	—	—	—	—	—	1♀	—	—	—	—	—	—	—	—	—	—	—	—	—
Tyroglyphus longior	—	—	—	—	—	—	—	—	1♀	—	—	—	—	—	—	—	—	—	—	—	—	—

M = *Mus musculus*; A = *Apodemus hebridensis*; B = Barvas, west coast; S = Stornoway, east coast; Nu = numerous in number; N_2 = deutonymphal stage; L = larval stage.

Family CHEYLETIDAE. Many specimens of *Myobia musculi* (Schrank) were found on one field mouse (No. 17) clinging to the hair on the head and neck. These mites are said to feed not on the blood of the host, but on exudates and skin debris.

Family LAELAPTIDAE. By far the greatest number of mites belonged to this family, which is one of the largest groups of mites. The commonest species was *Eulaelaps stabularis* (Koch), the female of which was found in small numbers on both field and house mice. Both males and females occurred in practically all the nests of *Mus musculus*, as many as forty-seven being present in one nest (No. 59). This species is often found in association with rodents and moles, the mites frequently forming large brown scales on the body of the host. The male is rarely present on the host, but is commonly seen running about the

Table II. *Samples of mites from nests of* M. musculus, *Barvas, Isle of Lewis.*

Ref. No. of tube ...	6	50	51	52	54	55	56	57	58	59
Total No. of mites ...	8	7	33	26	5	37	53	17	13	147
Species of mite:										
Eulaelaps stabularis	—	11♀, 1♂	8N_2, 16♀, 2♂	13♀, 4♂	—	24♀	8♀, 12♂	7♀	5♀	9N_2 7♂ 31♀
Ichoronyssus carnifex	3♂, 3♀	2♀	—	1♀, 16♂	—	7♀	19♀, 1♂	3♀	1♀	1♂, 2♀
Haemogamasus michaeli	1N_2	31♀, 11N_2	4♀	—	—	6♀	4♀, 2N_2	5♀	—	—
Gamasus sp.	1N_2	—	3♀	2N_2	—	—	3N_2, 1♀	1N_2	6N_2	6N_2
Myonyssus decumani	—	12♀, 5♂	—	—	3♂, 2N_2	—	—	1♀	1♀	32♀, 38♂, 15N_2
Androlaelaps pilifer	—	3♀	—	—	—	—	—	—	—	—
Veigaia cervus	—	—	—	—	—	—	2♀	—	—	—
Parasoides carabi	—	—	—	—	—	—	1N_2	—	—	—
Glycyphagus crameri	—	—	—	—	—	—	—	—	—	3♀
Tyroglyphus longior	—	—	—	—	—	—	—	—	—	1♂
Eugamasus cornutus	—	—	—	—	—	—	—	—	—	1♂

nest. Both sexes of *Eulaelaps stabularis* are frequently found gorged with blood, but they will also attack and suck the juices of other mites and small insects.

Myonyssus decumani (Tiraboschi) was found only in the nest of the house mouse where it was present in very large numbers. Both this species and *Myonyssus gigas* (Oudemans) are commonly found in rodent and mole nests in Great Britain, Germany and Holland. Small numbers of *Myonyssus gigas* were also found at Oxford in the fur of *Apodemus sylvaticus*, although the species is primarily nest-dwelling. The gut in males, females and nymphs was practically always distended with blood.

Laelaps festinus (Koch) was found only on the field mouse, although it has been recorded by Hirst (1916) as occurring on the house mouse in the Shetland and Orkney Islands. Two other species, *L. hilaris* (Koch) and *L. pachypus*

(Koch), that were commonly found on rodents in Bagley Wood, Berkshire, were not present in this collection.

Androlaelaps pilifer (Oudemans). Three female specimens of this mite were found in a heavily parasitised nest (No. 50) of *Mus musculus*. This mite has previously been recorded as living in moles' nests in Holland (Oudemans, 1931), although Hull (1925) has found another species (*Androlaelaps hermaphrodita* Berlese) free-living in the north of England. Moles are unknown in the Isle of Lewis, so one must assume that this mite, like many others, is also capable of inhabiting rodent nests.

Ichoronyssus carnifex (Koch). Males and females were collected from both the house and field mice. This mite, to the best of my knowledge, has not yet been found in England, although it has been found in large numbers on rodents collected by Mr Elton from Norwegian Lapland. It has also been recorded from Holland and Germany.

Family Haemogamasidae. The mites belonging to this family are similar in habits to the majority of the Laelaptidae. All are parasitic and commonly found on rats, mice, moles and other mammals. In the present collection, *Haemogamasus michaeli* (Oudemans) was found in the nest of the house mouse, although it has also been found by G. Bathurst Honey (Hirst, 1916) on *Apodemus hebridensis* in the Isle of Lewis.

Family Gamasidae. A considerable number of Gamasid deutonymphs were present in this collection, taken either from the house mouse or its nest. Many of these deutonymphs have been inadequately described and in some cases assigned by previous writers to the wrong species of adult; I have therefore avoided naming them. A few adults were also present, the identification of these however requires further study.

Family Tyroglyphidae. Three female specimens of *Glycyphagus crameri* (Michael) were also found in a nest of *Mus musculus*. The adults and nymphs have previously been recorded from moles' nests in England, Germany, Holland and Italy. I can find no previous mention of their occurrence on rodents or in rodent nests.

(B) *Casual inhabitants*

Family Ascaidae. Deutonymphs of *Asca affinis* (Oudemans) were found on both house and field mice and they are commonly present in the fur of many moles and rodents. They probably act as scavengers, since the gut never appears to contain any coagulated blood. The adults are unknown.

Family Gamasidae. Two deutonymphs of *Parasoides carabi* (Canestrini) were found in a house-mouse nest. This mite is commonly found attached in swarms to beetles of the genus *Necrophorus* which are used as a means of transport. As nearly related forms are parasitic on mammals, this mite may occasionally feed on the mice, although details of its feeding habits are unknown.

Two females of *Veigaia cervus* (Kramer) were taken from a house-mouse nest. The species has also been found on *Apodemus sylvaticus* in Bagley Wood and in moles' nests in Holland (Oudemans, 1931). I have also found both deutonymphs and females in decaying humus, feeding on the minute Arthropods that are found there. Neither of the specimens contained blood, and it is probable that these mites act as scavengers and live on other small parasitic Acari.

(C) *Accidental visitors*

Family Gamasidae. One male of *Eugamasus cornutus* (Canestrini) was found in a nest of *Mus musculus*. This is one of the commoner British Gamasids and can be found in practically any sample of mites taken from the surface soil. It has similar habits to *Veigaia cervus* but occurs more frequently free-living than in association with rodents. One female of *Cosmolaelaps claviger* (Berlese) was also found on a house mouse. This mite is also commonly found running amongst dead leaves and mould.

Family Oribatidae. One female of *Hermannia bistriata* was found in a mouse nest. As the Oribatidae are vegetable feeders, one may assume that its occurrence here was accidental.

Summary

Sixteen species of mites belonging to nine different families were found on *Mus musculus* and *Apodemus hebridensis* and in the nests of the former. Of these, eleven species may be regarded as parasitic in habit, two species have previously been recorded in association with rodents and moles, and three species may be described as accidental visitors.

I wish to thank Mr Elton and Prof. Munro of the Imperial College for their help, and I am also greatly indebted to Dr Calman and Dr Finnegan for allowing me to make use of the facilities afforded by the British Museum (Natural History).

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STUDIES ON THE INFLUENCE OF THE ENVIRONMENT ON THE SHEEP BLOW-FLY *LUCILIA SERICATA* MEIG.

I. THE INFLUENCE OF HUMIDITY AND TEMPERATURE ON THE EGG

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(With 8 Figures in the Text)

CONTENTS

I. Introduction

The results reported here are part of an investigation into the effects of various climatic factors which influence the life cycle of *Lucilia sericata* Meig. Graham Smith (1916, 1919) has studied the effect of climate on blow-flies under field conditions; in this and the following papers the problem will be considered rather from the laboratory point of view. *Lucilia sericata* is attracting world-wide attention because of its facultative habit of laying eggs on living sheep, the resulting myiasis causing enormous loss of time and money. Numerous records also exist of its occurrence in human myiasis. A knowledge of the effects of climate on all stages of the fly is most important in planning effective measures of control.

II. Humidity

Many workers have reported upon the effect of humidity on the eggs of insects which live in various habitats. Two examples may suffice to show how varied are the results obtained. Holdaway (1932), working with *Tribolium confusum* Duval, found that humidity had but little effect on the duration of the egg stage at a temperature of 27° C., and a very little effect on the viability of the eggs except at a very high relative humidity where development is prevented by the growth of fungi. On the other hand Janisch (1930), studying

Prodenia littoralis, found that the duration of development, percentage development and mortality were profoundly affected by the humidity of the surrounding air.

So far, however, a detailed study of the relations between humidity and any insect egg has not been made, and it is hoped that the following observations will help to fill this gap in our knowledge, which can only be filled adequately by a study of the different types of eggs known to exist.

(*a*) *Relation between humidity and duration of development*

The technique used in this study was as follows. Random samples of fifteen eggs from one freshly laid cluster were placed in orderly fashion on glass slides, suspended in air-tight glass jars over mixtures of sulphuric acid and

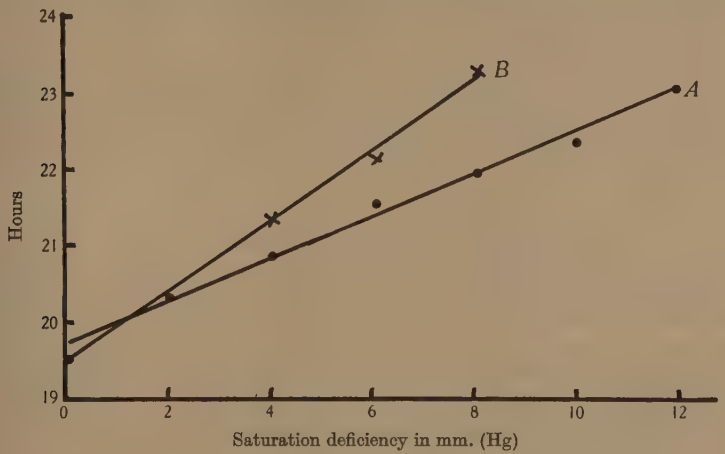

Fig. 1. The relation between humidity and duration of development.

water giving the required humidities, and placed in a constant temperature room at 22° C. The time of laying was recorded as the mean between the time at which the first and last eggs were laid. The time of hatching of each egg was recorded to within 10 min. When the eggs kept at 0 mm. saturation deficiency had hatched, the remaining experimental batches were transferred to jars containing distilled water to eliminate the retarding effect of humidity upon hatching, which occurs in this species and was also found in *Prodenia littoralis* by Janisch (1930).

A result obtained in this way is given in Table I and Fig. 1, *A*. From these it will be seen that the relation between humidity and duration of development is linear. The durations of development calculated from the formula

$$y = 0{\cdot}29x + 19{\cdot}6, \qquad \ldots\ldots(1)$$

where y = duration of development and x = humidity expressed as saturation deficiency, agree closely with those found experimentally.

Table I. *Relation between duration of development expressed in hours and humidity expressed in mm.* (Hg) *saturation deficiency.*

Saturation deficiency	Duration of development found	Calculation
0	19·5	19·6
2	20·3	20·2
4	20·8	20·7
6	21·5	21·3
8	21·9	21·8
10	22·3	22·4
12	23·0	23·0

One batch of eggs was sufficiently large to run a duplicate experiment and allow one series of eggs to hatch at the humidity at which they developed. The retarding effect of low humidity on hatching is clearly seen in the upper curve (*B*) of Fig. 1.

Cousin (1932) reports that the rate of development of eggs kept in water is retarded, but this observation has not been confirmed, the duration of development of eggs covered by a thin film of water being similar to that of eggs kept in saturated air.

(*b*) *Relation between humidity and loss of water*

The question now arises as to how this retardation in velocity of development is brought about. The first obvious explanation is through a loss of water slowing up in one way or another the processes of development. Buxton (1932) has suggested that Bělehrádek's theory on the influence of viscosity on vital processes may apply here. Fig. 2 shows the progressive loss in weight of several batches of eggs subjected to different humidities. Clearly, the lower the humidity the greater is the loss in weight. Later it will be shown that a constant amount of dry matter is consumed during development at different humidities, so that the differences shown here are due to loss of water.

By subtracting the loss of weight at 0 mm. saturation deficiency from the loss of weight at other humidities, the loss of water due to the evaporative power of the air is obtained. Analysis of the results so obtained shows:

(*a*) That the developmental period may be divided into three parts, the first lasting for about one-sixth of the total period, during which water is lost most rapidly; the second lasting for about two-thirds of the total period, during which water is lost less rapidly; and the third during which it is lost very slowly compared with the rates of loss for the other periods.

(*b*) That the lower the humidity the greater is the rate of loss of water over the whole developmental period.

(*c*) That equilibrium between the water vapour pressure of the air and the water vapour pressure of the colloid materials in the egg and developing larva is not reached before the eggs hatch.

At 18 mm. saturation deficiency the rate of loss of water follows a normal course until the ninth hour, when it accelerates rapidly. Eggs kept at this humidity do not complete their development. It is suggested that this sudden acceleration in rate of water loss is due to a change in the structure of the egg brought about by death.

Several attempts have been made to determine what relationship exists between loss of water and humidity at constant temperature, but it has been found difficult to draw any definite conclusions from the results obtained since these are somewhat erratic. An explanation of these erratic results will be

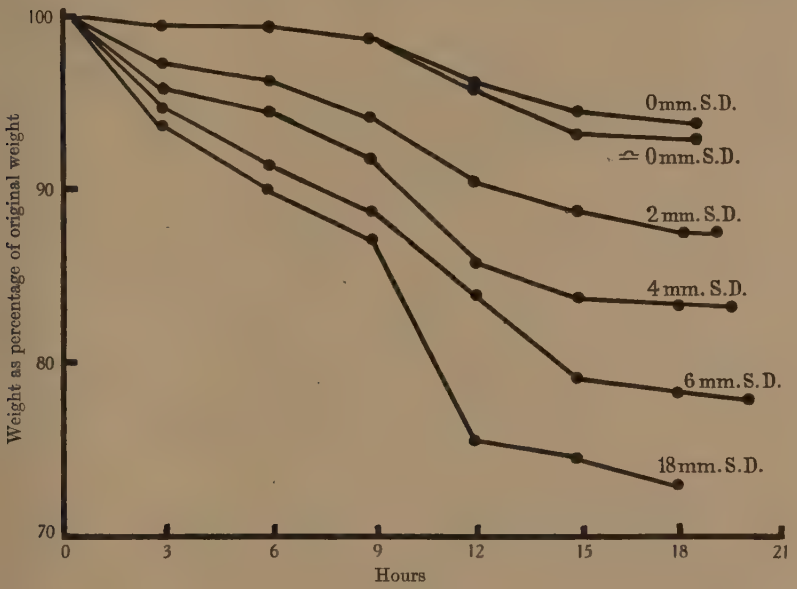

Fig. 2. Progressive loss of weight at different humidities.

given below in discussing the influence of percentage dry weight on the loss of water. Two sets of results, however, suggest that a linear relationship exists between the amount of water lost and humidity. One set of results is given in Fig. 3, showing the loss of weight at periods of 1·5, 3·0 and 4·5 hours. The dry weights of the batches used were fairly comparable, being 27·3, 27·0 and 28·4 per cent. at saturation deficiencies of 4, 12 and 20 mm. respectively. If reliance can be placed upon these results it will be seen, comparing Figs. 1 and 3, that there is a direct relationship between the retardation in velocity of development and the amount of water lost by the eggs.

(*c*) *Relation between loss of water and dry weight*

While the above experiments were being carried out, it was sometimes found that a single batch of eggs kept at a low saturation deficiency lost appreciably more weight than a batch kept at a high saturation deficiency. It had previously been noticed that the percentage dry weight of the eggs varied. This suggested a relationship between the amount of water initially present and the amount of water lost under constant conditions of humidity, temperature and time. Fig. 4 shows that the relation existing between the amount of water

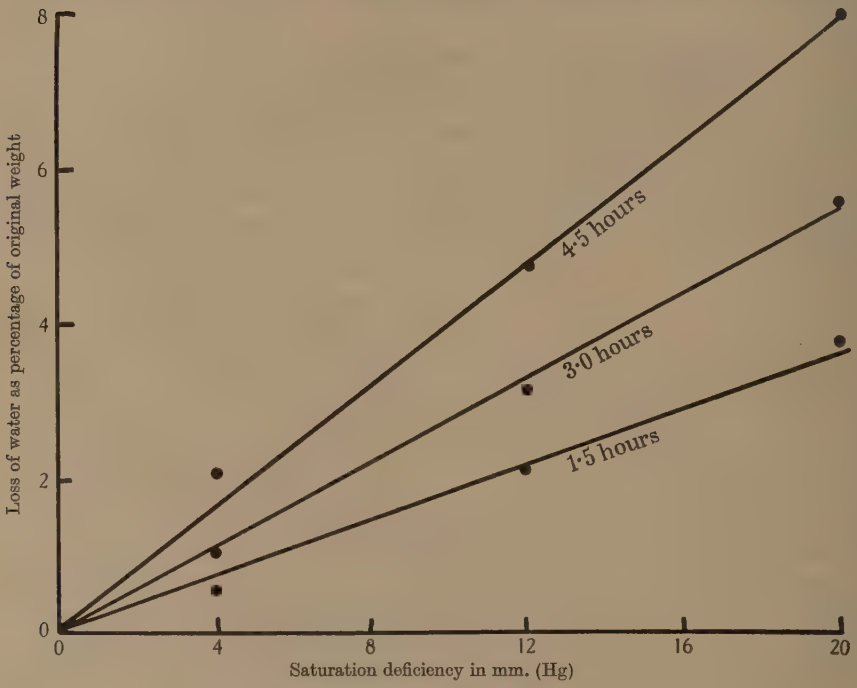

Fig. 3. The relation between humidity and loss of water.

initially present and the amount lost under stated conditions is linear. The two graphs are from two separate batches of flies and show that other factors beside the initial water content or percentage dry weight are operative.

(*d*) *Regulation of loss of water by other factors*

The chorion plays a very definite part in governing the rate at which water is lost in dry air and also the amount of water which may be lost. This can be shown by dividing a large batch of freshly laid eggs into two portions, setting aside one batch as a control and splitting the chorion of each egg of the other

batch so as to expose the vitelline membrane, then exposing each batch to the same conditions of temperature and humidity. The experimental batch is found to lose water far more rapidly than the control batch and also loses a larger amount.

Table II shows the effect of damage to or removal of the chorion upon development of eggs at various humidities. Undamaged eggs survive in much drier air than eggs stripped of their chorion or possessing a broken chorion. Evidently this increased mortality of treated eggs is brought about by the increased loss of water shown in the preceding paragraph.

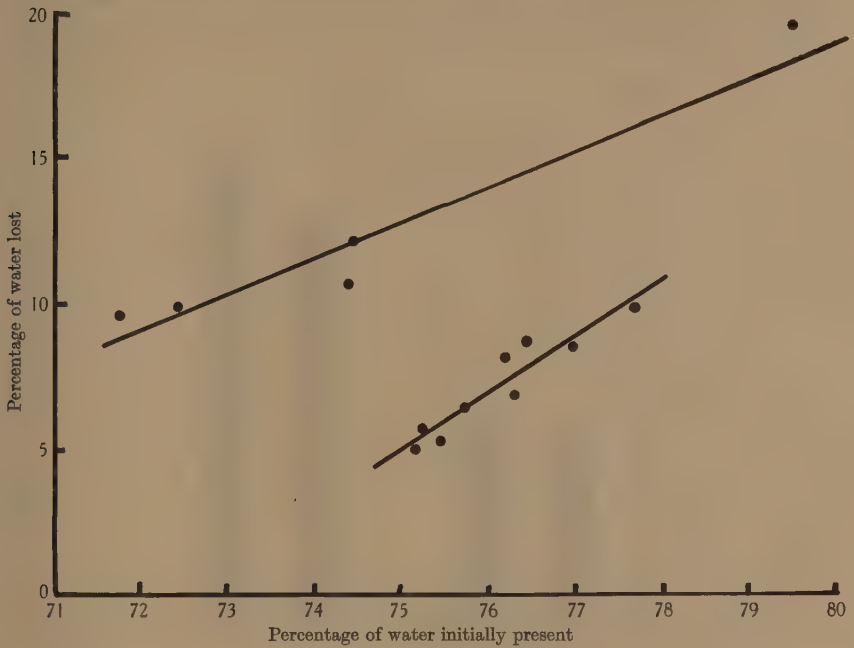

Fig. 4. The relation between loss of water and the amount of water initially present.

Table II. *Influence of the chorion on development at various humidities*, 23° C.

Relative humidity %	Control		Chorion broken		Chorion removed	
	Developed	Dead	Developed	Dead	Developed	Dead
100	14	1	15	0	15	0
90	15	0	8	7	0	15
80	13	2	2	13	0	15
70	15	0	0	15	0	15

The vitelline membrane also plays a part in governing the loss of water. If a freshly laid egg be stripped of its chorion it will maintain its characteristic shape for several hours when exposed on the laboratory bench, but if one end

of an egg so treated be pricked, that portion of the contents which flows out soon dries up.

It can be shown that temperature is an important factor in governing the loss of water from the egg by measuring the loss in weight of a batch of eggs at a constant saturation deficiency first at a low temperature, then at a high temperature for equal periods of time. The conditions chosen for this work were saturation deficiencies of 10 and 6 mm. (Hg) at temperatures of 14 and 22° C. Under constant conditions of temperature and humidity the rate of loss of water decreases with time but Fig. 5 shows that, at a constant saturation deficiency, the rate of loss of water increased with an increased temperature during the period when it would have decreased had temperature not been a factor.

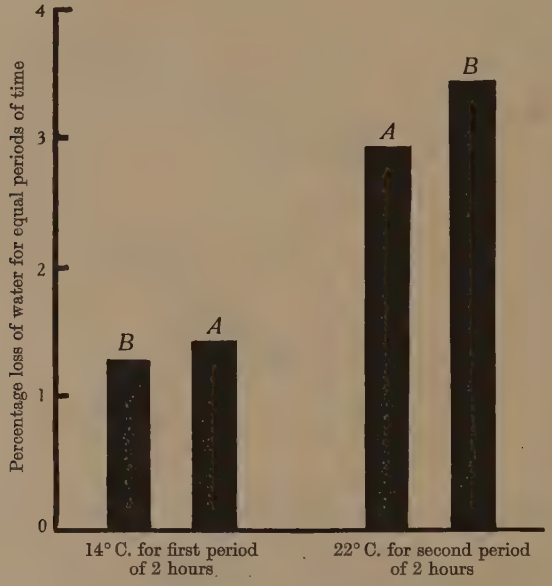

Fig. 5. The influence of temperature on water loss. *A* at 10 mm. saturation deficiency; *B* at 6 mm. saturation deficiency.

(*e*) *Irreversibility of water loss*

Several investigators have shown that eggs of insects can, under certain conditions, imbibe large quantities of water. This imbibition of water is a normal occurrence in the development of the egg of *Anisoplia austriaca* (Col.) and of certain sawflies (Kerenski, 1930). In the case of a grasshopper, it occurs when eggs which have been kept under arid conditions are placed in a moist environment (Parker, 1930).

It has been found that although the eggs of *Lucilia* lose water readily under dry conditions, they will not absorb water if replaced in a saturated atmosphere.

This is clearly shown in Fig. 6. Batches of eggs were subjected alternatively for hourly periods to humidities of 10 mm. saturation deficiency and of 0 mm. saturation deficiency with one control at 10 mm. saturation deficiency continuously. The figure demonstrates clearly that in the drier atmosphere the eggs lost weight, while in the saturated atmosphere their weight remained constant.

Batches of normal eggs and of eggs subjected to dry air for short periods were soaked in water for varying periods, but no evidence of any absorption of water was obtained. Also, eggs which had been dried almost to the lethal limit and then soaked in water showed a similar mortality to controls kept in saturated air, from which it has been conclusively demonstrated that no absorption occurs (Table III).

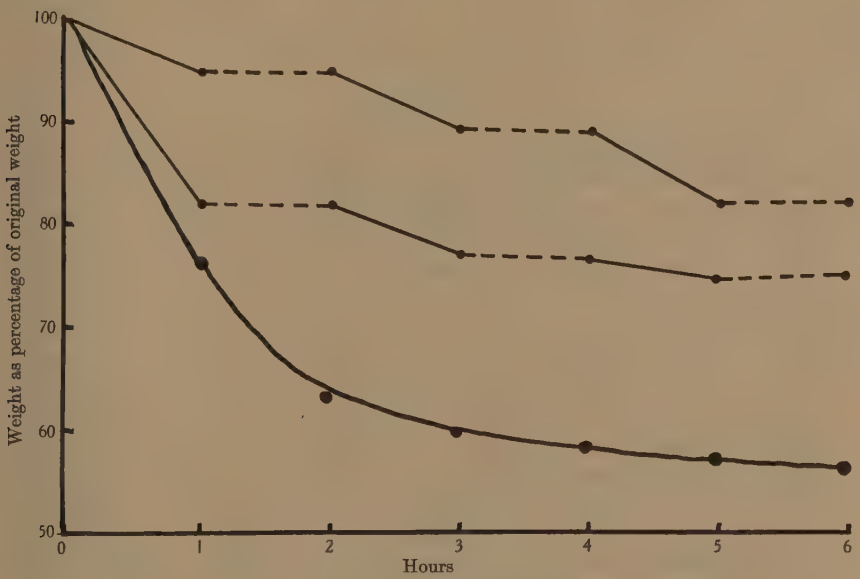

Fig. 6. Irreversibility of water loss. —— at 0 mm. saturation deficiency; - - - - - at 10 mm. saturation deficiency.

Table III

	Percentage survival of dried eggs in	
	Saturated air	Water
I	19	12
II	15	19

(*f*) *Consumption of dry matter at different humidities*

It has been shown in the case of *Tenebrio* that the larva of this insect is able to regulate its water content at different humidities by increased combustion of reserve materials, chiefly fat (Mellanby, 1932). It is of importance to know

whether the egg of *Lucilia* can regulate to any extent its water content and so increase its resistance to adverse conditions of humidity.

Batches of eggs were divided into two portions and weighed. One portion was incubated at a humidity of 0 mm. saturation deficiency, the other at a humidity of 10 mm. saturation deficiency. Just before the eggs were about to hatch they were reweighed and their dry weights estimated. Table IV gives the percentage dry weights calculated on the initial weight. It will be seen that there is no significant difference between the two sets of eggs.

Table IV. *Dry matter expressed as percentage of original weight.*

	Saturation deficiency		
	0 mm.	10 mm.	Difference
A	25·0	25·5	+0·5
B	22·2	22·2	0·0
C	23·1	22·9	−0·2

III. Temperature

The effect of temperature on rate of development has been discussed by Wardle (1930) and Cousin (1932), who show that for median temperatures the relation between the length of development and temperature is the characteristic hyperbola commonly found in investigations of this type. Unfortunately no attention has been paid, to my knowledge, to the effect of limiting temperatures on this relationship.

The effect of temperature on hatching in saturated air may be gathered from Fig. 7. An 100 per cent. hatch occurs over a wide range of temperatures, from 14 to 35° C. Mortality rapidly increases with a comparatively small increase or decrease in temperature to limiting temperatures of about 10 and 40° C. It may be noted here that Wardle (1930) describes hatching at 10° C. and Cousin (1932) hatching at 40° C. with a remark that this temperature is "trop haute," presumably for an 100 per cent. hatch. An 100 per cent. development occurs over a slightly larger range of temperature than hatching, 14–37·5° C., with limiting temperatures of approximately 8 and 40° C. at which no development occurs.

IV. Temperature and humidity in combination

Perhaps the most interesting study is that of two or more environmental factors upon a phase in the life cycle of an insect. Buxton (1931) has discussed the effect of temperature and humidity in combination on the egg of a grasshopper, *Melanoplus atlanis*. He showed that, over a wide range of temperature, mortality was governed by humidity expressed in terms of saturation deficiency. The relationship is strikingly simple—25 per cent. of the eggs died at a saturation deficiency of 5 mm., 50 per cent. died at a saturation deficiency of 10 mm.

Fig. 7 shows the effect of humidity and temperature in combination upon

the survival of the egg. Clearly, the relationship between humidity and survival is not as simple in the case of *Lucilia* as in the case of *Melanoplus*. At low and at high temperatures a low saturation deficiency will bring about death, while at median temperatures higher saturation deficiencies can be tolerated. Thus with humidity as a factor the range of optimum temperature is greatly reduced, being 27–32° C.

The important factor governing mortality is loss of water and not temperature directly. This can be seen from the wide range of temperatures at which an 100 per cent. hatch may be expected in a saturated atmosphere. Loss of water from normal eggs is governed chiefly by the variable factors temperature,

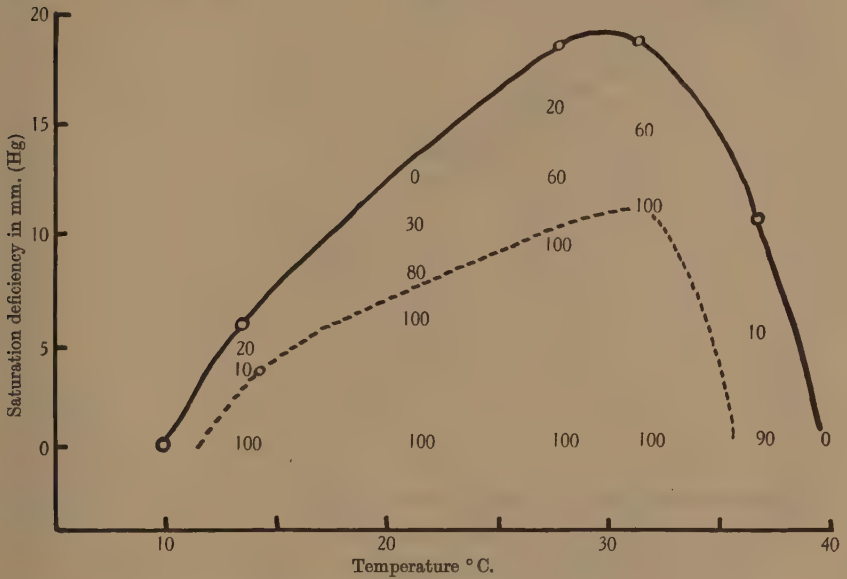

Fig. 7. Mortality limits at different combinations of temperature and humidity. Figures represent the percentage of eggs which hatch.

saturation deficiency and time of exposure. The actual lethal times of exposure to combinations of temperature and humidity, which just cause 0 per cent. hatching, have not been determined. As an approximation to this period, the duration of the egg stage at a slightly lower saturation deficiency (*i.e.* a humidity at which some eggs do hatch) has been taken. Considering the conditions of temperature, humidity and time which entirely prevent hatching, it is found that these three variables can be related to each other in a simple and direct manner by means of the formula

$$y = m(x \times z) + c, \qquad \ldots\ldots(2)$$

where y = temperature, x = saturation deficiency, z = time of exposure. The quotient $x \times z$ is an expression of the effect of humidity on the egg. The linear

relationship existing between the expression $x \times z$ is shown in Fig. 8. In other words, the higher the temperature the more susceptible is the egg to equal evaporative conditions. This is clearly due to the greater loss of water at high temperatures compared with that at low temperatures and equal saturation deficiencies. How this is brought about the writer does not profess to explain, but its importance should be emphasised. Entomologists, in seeking for an expression of humidity independent of temperature, have seized upon saturation deficiency and for several sets of data it has proved to be the important

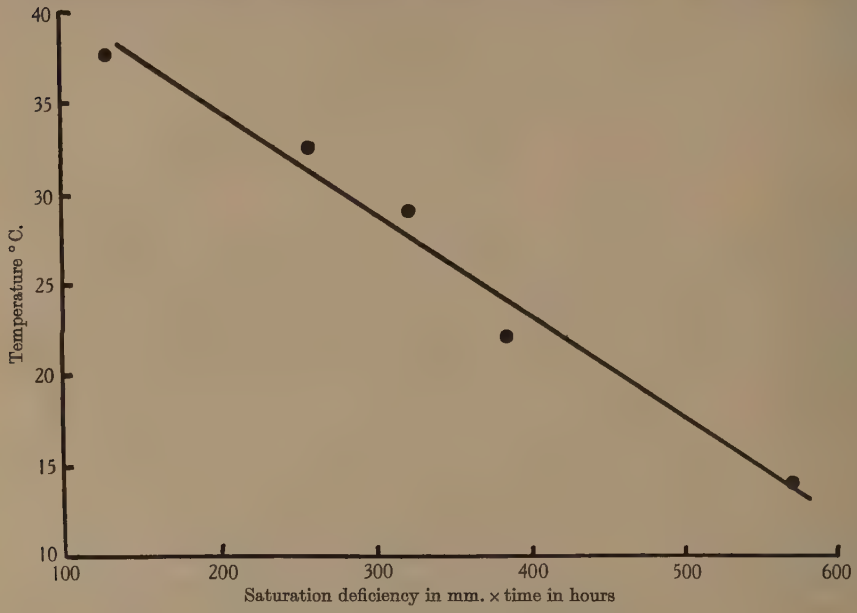

Fig. 8. Relationship between temperature, humidity and time leading to failure to complete hatching.

factor, *i.e.* limiting length of life of adults or determining percentage hatching of eggs. Unfortunately exceptions to useful generalisations will occur and this seems to be a case in point.

V. Summary

The many factors governing loss of water from the egg of *Lucilia sericata* are discussed, and this loss of water is shown to be the probable cause of mortality and failure to complete development at different combinations of temperature and humidity.

I am greatly indebted to the Agricultural Research Council, by whom the cost of this investigation has been defrayed, and to Prof. P. A. Buxton for his helpful advice and criticism.

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THE TACHINID PARASITES OF WOODLICE

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(With Plates XV–XXII, containing Figs. 1–79, and Figs. I–V in the Text)

CONTENTS

I. Introduction

Whether we hold, with the Lamarckians, that evolution is, in the main, the result of purposeful action, or with the Darwinians, that it represents merely the effect of the hazards of existence on random variations, we must admit that it has occurred only within rather narrow limits. Research has revealed a multitude of variations on the main themes of organic nature; but for many years it has discovered neither any new themes, nor any transitions between those with which we were already familiar. This seems to be true not only of plans of structure but also of environmental or ecological groups. The line of the high tides marks, between the rich and varied life of the sea and the meagre

fauna of the land, an almost impassable barrier. It is a curious fact that even among such dominant terrestrial groups as the insects, abounding in both species and individuals, secondary adaptations to marine life are almost completely absent.

This infrequency of migrations from the land to the sea, and from the sea to the land, limits very strictly the opportunities of contact between marine and terrestrial animals; and this is no doubt why parasitic insects have never taken advantage of the vast mass of potential hosts present in the sea. Within the limits set by instinct, the choice of hosts by parasites appears to be very largely determined by propinquity. Many species that might be chosen escape, not because they are intrinsically unsuitable or distasteful, but merely because they are unattainable. The parasitic Diptera and Hymenoptera confine themselves almost entirely to terrestrial insect hosts, but since the entomophagous forms have presumably arisen from phytophagous stocks and have passed from a vegetarian to a carnivorous régime, it is difficult to see why they should restrict themselves so closely to an insect diet, except that insects were the most common and accessible prey.

The rather curious case studied in this paper appears to confirm the foregoing views. In spite of the abundance and variety of existing Crustacea, parasitic insects very seldom attack the members of this group. The Crustacea have, however, produced an insignificant band of adventurers that has moved up on to the land—the terrestrial Isopoda, or woodlice. This migration has made the crustaceans available to parasitic insects, and the latter have responded by producing the curious group of forms described in this paper. It has seemed worth while to record this unusual case of parasitic adaptation in some detail.

II. HISTORICAL

The earliest reference to the dipterous parasites of woodlice that I have been able to find in the literature is contained in a paper by v. Roser (1840), dealing with the Diptera of Württemberg. In this paper, v. Roser records a species cited under the name of *Tachina atramentaria* as a parasite of a terrestrial Isopod, doubtfully determined as *Oniscus asellus* L. However, the specimen does not seem to have been examined by any competent specialist. Nielsen (1909), in his review of Tachinid biology, quotes Brauer and v. Bergenstamm (*Zweifl.* VII, p. 2) as referring to the rearing of *Stevenia umbratica* from *Oniscus asellus* by v. Roser; but no such statement is to be found in the works of these authors, and it seems evident that the reference to *Stevenia umbratica* is due simply to a mistake made by Nielsen in transcribing the reference. Other authors, as for example Lundbeck (1927) and Baer (1920–1), have apparently copied the reference to *Stevenia umbratica* from Nielsen, so that we have in reality no evidence that this species was ever reared from woodlice.

No further mention of the parasitism of woodlice by Diptera occurs until 1903, when C. T. Brues published in *Entomological News* a paper recording the

rearing of *Melanophora roralis* L. from a species of *Porcellio* (probably *P. scaber* L.) found near Woods Hole, Massachusetts.

The next record is due to Donisthorpe, who reared, from specimens of *Oniscus asellus* L. collected at Bembridge in the Isle of Wight, in 1908, two specimens of *Phyto melanocephala* Meig., which are now in the collection of the British Museum (Natural History), together with one of the dead woodlice, containing an empty puparium. According to Wainwright (1928), Donisthorpe also reared *Ptilocerina atramentaria* from *Oniscus asellus*, but Mr Donisthorpe informs me that this is incorrect.

In the work of Baer (*loc. cit.*) and Lundbeck (*loc. cit.*), *Frauenfeldia rubricosa* is stated to be a parasite of *Oniscus asellus*, but I have not been able to trace the source of this information. In 1931, E. O'Mahoney reared this species from *Porcellio scaber* L.

The present writer began work on the parasites of woodlice early in 1917 when stationed at the R.N. Hospital at Haslar, near Gosport. Collections were made without success in the Isle of Wight, but a little later a rich supply of material was found in a colony of *Porcellio* and *Oniscus* living under the bark of a fallen tree in the grounds of the hospital, and eventually one parasitised specimen was actually found in the cellar of the clinical laboratory, in which the writer was then working. A preliminary note summarising the results of this work was published in the autumn of 1917. Later investigations showed, however, that this short note contained several errors. The material described as one species actually comprised larvae of two forms, neither of which belong to the species (*Phyto melanocephala* Meig.) with which they had been identified. In a later note, published in 1920, the writer recognised the existence of at least two species in the material, and suggested that the first-stage larvae were those of *Phyto melanocephala*, while the later stages were those of *Melanophora roralis* L. Further investigations and comparisons showed, however, that both of those statements were erroneous. The specimens actually reared were subsequently identified by Dr Villeneuve as *Styloneuria discrepans* Pand., and *Frauenfeldia rubricosa* Meig.

In the winter of 1919–20, while working in the Department of the Gers, in south-western France, the writer collected specimens of an Isopod not hitherto examined, which was determined by Dr Racovitza as *Metaponorthus pruinosus*. In some of these there were found larvae of a Tachinid differing markedly from those hitherto observed, and of which the adult was determined by Dr Villeneuve as *Cyrillia angustifrons*, a species originally described by Rondani, but so rare that Dr Villeneuve himself had seen, up to that time, only a single specimen (collected at Sion, Valais, Switzerland) in any European collection. A brief summary of the biology of *Cyrillia* was published in the *Comptes rendus de l'Académie des Sciences* in 1920.

Since that time the investigations have been continued as opportunity offered. The Mediterranean littoral, on which the writer spent the next few years, is, however, unsuitable for woodlice, because of the semi-arid conditions

that prevail during the greater part of the year. A small supply of material, containing larvae of *Frauenfeldia rubricosa* and an undetermined species, was received from the late Miss Marie Rühl of Zurich, Switzerland, but it was not until the writer returned to England in 1928 that it was possible to make any real progress with the work.

The study of these parasites has proved quite exceptionally difficult and laborious. The proportion of woodlice attacked by all the species taken together seldom exceeds 5 per cent., and is usually below that figure. None of the species studied passes through more than a single generation per annum. The larvae are very similar, especially in the second stage, which is the one in which they are most frequently found in dissections. In such cases, the mouth-hook of the preceding stage, which in many Tachinids remains attached to the posterior extremity of the second-stage larva, is often of great help in the separation of species; but in the parasites of woodlice it is very difficult to find, partly because it is relatively feebly chitinised, partly because it very often becomes detached from the body of the larva. Finally, as we shall have occasion to show later in the paper, the females of these parasites, unlike the majority of Tachinids, are oviparous, depositing eggs which resemble those of the ordinary blow-fly, but require a considerable period for their development so that the dissection of females is of no assistance in working out the life history. Nevertheless, with the help of Mr S. Kozlovsky, a large collection of the larval forms was finally obtained for study, and although a number of points still remain to be cleared up, the life history of the majority of the species of the group has been worked out.

III. The systematic position of the parasites of woodlice

The investigations on the biology of the Tachinidae and their allies that have been carried on during the last twenty years, have made it clear that the systematic arrangement of these forms, based upon the external anatomy of the adults, upon which the majority of dipterologists still rely for their determination of affinities, does not by any means agree with the arrangement based on the reproductive habits and the structure of the eggs and larvae. Thus, as the writer pointed out some years ago (1926), the genus *Exorista*, established on adult characters, contains some species depositing, on the body of the host, a plano-convex macrotype egg, without a pedicel but with a hard resistant shell, and containing an undeveloped embryo (group I of Pantel), others depositing, on the food plant of the host, microtype, hard-shelled eggs destined to be swallowed and containing a larva ready to hatch, still others depositing on the body of the host a larva, which hatches during oviposition or immediately afterward, the egg being thin-walled and flexible, and, finally, a group of species depositing, on the body of the host, eggs bearing, at the posterior pole, a stiff cylindrical stalk terminating in an adhesive expansion by which the egg, which contains a well-developed larva, becomes fixed to the skin of the prey.

Some students of Tachinid biology, as for example the late Père Pantel, S.J., are of the opinion that a grouping based upon reproductive habit and the characters of the early stages has little or no taxonomic significance. For these authors, the resemblance in the structure of the eggs and larvae is merely the results of an evolutionary convergence, having as its point of departure the similarity in reproductive habit, and can be of no assistance in determining the true systematic affinities within the group.

It does not seem, however, that there is any solid basis for this view. It is just as easy to imagine that the differences in reproductive habit and larval structure are primary and the differences in the conformation of the adults secondary as it is to imagine the reverse; especially in a group where the generic characters are so superficial and so ill-defined as they are in the Tachinidae. In point of fact, we possess no solid evidence in favour of either view and no good reason to prefer one to the other. It is, no doubt, preferable, for reasons of convenience, to establish a system of determination based upon adult characters, rather than on those of the larvae; but we have no reason to suppose that the adult characters indicate more clearly than those of the early developmental stages the systematic affinities of the various forms. It would seem, on general grounds, that to establish a really satisfactory classification, a thorough knowledge of the organism in all the stages of its development is not only desirable but necessary.

It appears, however, that some of the systems of classification proposed by more recent students of the Muscoid Diptera are rather more in agreement with the results obtained from the larval stages than those of their predecessors. Within the subfamily Tachininae no very satisfactory correspondence is noticeable; but the Dexiinae, the Sarcophaginae, and the Phasiinae contain many of the same elements, whether we define them on reproductive habit and early stage characters, or on adult structure. Finally, the group of the Rhinophorinae, as defined by Girschner and Villeneuve, actually contains all of the parasites of woodlice of which the identity is known. All of the genera containing species with this habit are classed by Dr Villeneuve (1924) in the section *Rhinophora*, which contains a number of other genera (*Rhinomorinia*, *Morinia*, *Anthracomyia*) depositing eggs similar to those of species treated in this paper, though their biology is unknown.

Finally, it must be noted that in the generic arrangement adopted by Wainwright, in his excellent and useful paper on the British Tachinidae, all of the dipterous parasites of woodlice known to exist in this country fall together at the end of the subfamily Tachininae, although, so far as I am aware, Mr Wainwright had no information as to the biology of the majority of these species. We may therefore hope that a more intensive study of the adult characters, carried on in conjunction with those to be drawn from a study of biology and larval structure, will eventually lay the groundwork of a system of classification in which the true natural affinities are expressed and which is taxonomically valid as well as useful.

As an examination of the descriptions and figures in this paper will show, the parasites of woodlice, whose development is known, may be classified according to larval structure into three groups. The first of these comprises the genera *Melanophora*, *Styloneuria*, *Phyto*, and *Plesina*; the second, the genera *Cyrillia*, *Frauenfeldia*, and the undetermined species B; and the third, the remarkable but still undetermined larva with the double anterior hooks. It may be noted that the apparently single anterior tooth of the first-stage larvae of the first group is actually composed of two sclerites closely appressed and partially fused, and that the existence of the double anterior hook in the species of the first and third groups indicates an affinity with the genus *Sarcophaga* and some closely related forms which these Rhinophorines also resemble in possessing in the larval stage a well-marked dorsal oesophageal caecum. Neither of these characters exist in any of the Tachininae or Dexiinae that I have so far examined. On the other hand, the habit of depositing eggs with a thin-walled, flexible shell, containing an undeveloped embryo which requires several days before it is ready to hatch, is a distinctive feature of these flies, marking them off from all of the other members of the Tachinidae, though judging from my dissections of adult females it occurs in a number of other Rhinophorine genera.

The separation of the Rhinophorines known to parasitise woodlice is not difficult, as their characters are well marked and clearly defined. The following table may be used for identifying the species dealt with in this paper:

I. First posterior cell of wing closed in the margin, but not stalked; wings hyaline; abdomen without discal macrochaetae (Figs. I_3, II_3). *Frauenfeldia rubricosa* Meig.

II. First posterior cell of wing with a short stalk, less than one-third the length of the apical part of the fourth longitudinal vein.

Abdomen without discal macrochaetae; wings hyaline; bend of fourth longitudinal vein sharply angular (Figs. II_1, III_4). . . . *Styloneuria discrepans* Pand.

Abdomen with discal macrochaetae. Bend of fourth longitudinal vein rounded; body black, only slightly dusted with pollen; wings hyaline. *Cyrillia angustifrons* Rond.

Bend of fourth longitudinal vein sharply angular; body grey, heavily dusted with pollen; wings hyaline (Figs. I_4, III_1). . . . . *Phyto melanocephala* Meig.

III. First posterior cell of wing with a long stalk, at least half as long as the apical part of the fourth longitudinal vein.

Wings pigmented, at least in part.

Arista bare (Figs. I_2, II_4). . . . . *Plesina maculata* Fall.

Arista plumose (Figs. II_2, II_5). . . . . *Melanophora roralis* L.

Wings hyaline.

Abdomen with discal macrochaetae (Fig. III_2). *Ptilocerina atramentaria* Meig.

Abdomen without discal macrochaetae.

Black, shining species; sides of the face bearing only short, fine hairs. (Figs. I_1, III_3). . . . . *Rhinophora lepida* Meig.

Pollinose species; sides of the face opposite the lower end of the eye, with several long, strong macrochaetae. . . . . *Stevenia umbratica* Fall.

It must be noted that only a single species belonging to each of the genera named has actually been reared. According to Wainwright, only one species of

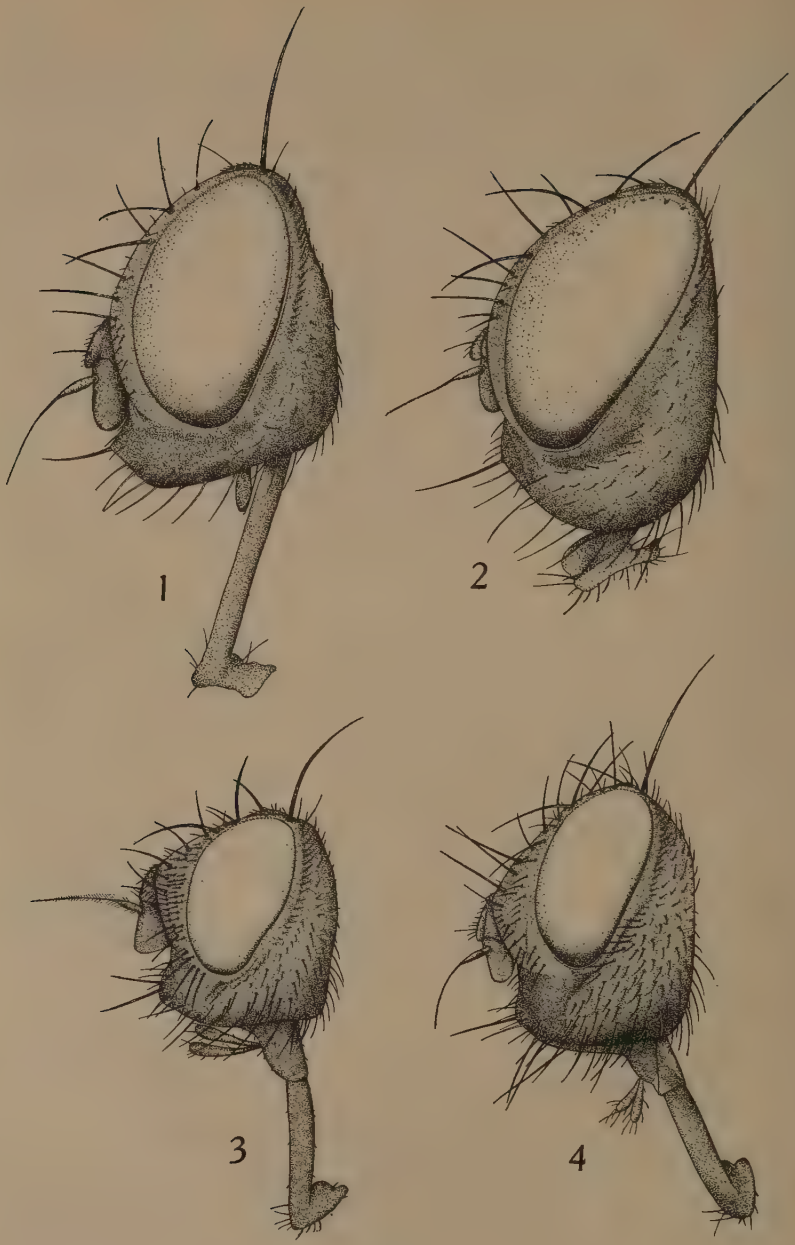


Fig. I.

1. *Rhinophora lepida* Meig. Head of adult, lateral view. ×50.
2. *Plesina maculata* Fall. Head of adult, lateral view. ×50.
3. *Frauenfeldia rubricosa* Meig. Head of adult, lateral view. ×33.
4. *Phyto melanocephala* Meig. Head of adult, lateral view.

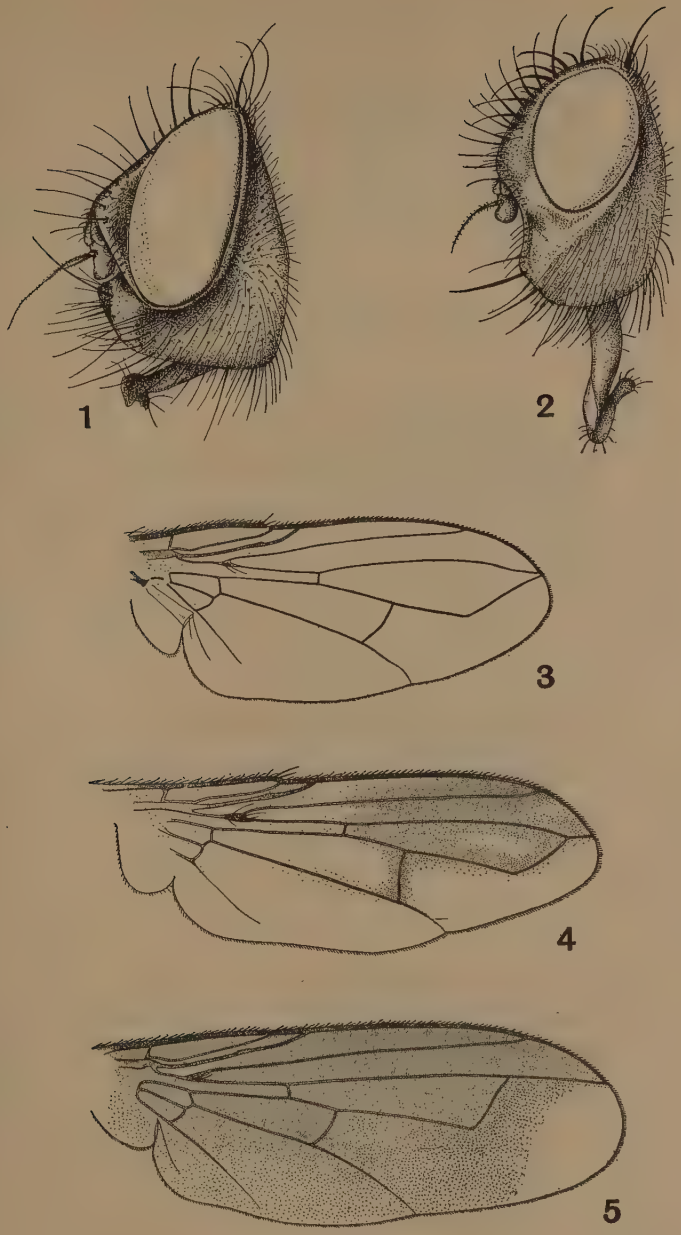
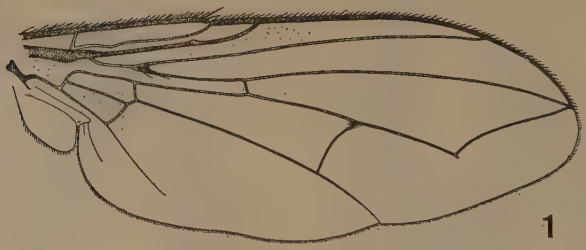


Fig. II.

1. *Styloneuria discrepans* Pand. Head of adult, lateral view
2. *Melanophora roralis* L. Head of adult, lateral view.
3. *Frauenfeldia rubricosa* Meig. Wing. ×25.
4. *Plesina maculata* Fall. Wing. ×19.
5. *Melanophora roralis* L. Wing, female. ×33.



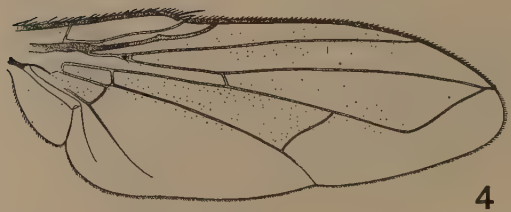


Fig. III.

1. *Phyto melanocephala* Meig. Wing. ×25.
2. *Ptilocerina atramentaria* Meig. Wing. ×25.
3. *Rhinophora lepida* Meig. Wing. ×25.
4. *Styloneuria discrepans* Pand. Wing. ×19.

each of these genera is actually known in Britain; but Bezzi and Stein list three species of *Cyrillia*, thirteen of *Phyto*, eight of *Plesina*, and five of *Melanophora*, as well as sixteen of *Stevenia* s.str. and nine of *Ptilocerina*; and though these may not all be "good" species, some of them—as, for example, *Plesina parmensis*—are undoubtedly distinct. A great deal more laborious rearing work with woodlice from all parts of Europe will have to be done before we can offer an adequate treatment of the group on larval characters.

IV. The larval forms of the parasites of woodlice

(*a*) Plesina *group*

(1) *Plesina maculata* Fall.

Stage I. Dimensions: length 0·45 mm., width (larva somewhat flattened by the cover slip) 0·114 mm. at widest point.

The larva (Fig. 5) is elongate but rather markedly spindle-shaped in form, increasing gradually in diameter from the anterior extremity to about the middle of segment VI, and from thence decreasing in diameter to the posterior end. It is composed of the usual rudimentary *head*, or *pseudocephalon*, *three* thoracic and, apparently, *nine* abdominal segments, the additional segment being produced apparently by a secondary constriction in the middle of the last segment, separating an anterior portion similar in its cuticular armature and sensorial organs to the rest of the abdomen from a posterior portion which is very different in structure; on the dorsal side, however, the two halves of the segment are not quite so distinct.

The larva resembles superficially those of the Echinomyiine and Dexiine groups, its closest morphological relative being, perhaps, the primary larva of the earwig parasite *Digonochaeta setipennis*; but it presents a number of very unusual features. It is characterised by a very well-developed pigmented cuticular armature (Fig. 10), which is, however, composed neither of groups of nodules, plates, or scales, as in the Echinomyiines and Dexiines, but of small rounded convex protuberances, which bear what on first examination seem to be groups of nodules, as in the larvae of *Echinomyia*, *Peleteria*, etc., but are in reality groups of short, stiff, vertical spines, of which one, occupying a central position on the protuberance, is usually much more developed than the others.

The *head*, or *pseudocephalon*, is of the usual type, much reduced in size and very simple in structure. Ventrally it is bare, but the dorsal and pleural regions bear minute, short, rounded nodules, rather feebly pigmented. Anteriorly the head bears a pair of well-developed, elongate, slightly curved, cylindrical *antennae* (length 0·0112 mm.), subacute at the tip, about seven times as long as wide and inserted on a rather broad, subconical, truncate base, which may be interpreted as a proximal segment, though it is not distinctly separated from the rest of the head (Fig. 2). Segment I bears, dorsally and pleurally, five or six irregular rows of papillae, bearing a short acute, slightly hooked spine,

surrounded by minute, nodule-like bristles; on the ventral surface is an anterior band composed of three irregular rows of broad, flattened, triangular spines, rather feebly pigmented, and a posterior band composed of two or three rows of short, acute, feebly pigmented spines, posteriorly directed and interspersed with small nodules. Segment II bears dorsally and pleurally six or seven rows of the typical papillae bearing a short, feebly pigmented but stout central spine, surrounded by a group of short, stiff bristles. On the anterior part of the ventral surface these are replaced by broad, flat, feebly pigmented scales, triangular in form, while the median part of the posterior border is bare. Segments III–X are similar in structure, the greater part of the surface being covered with the spinose papillae already described, which are, however, replaced along the ventral conjunctivae by bands of acute, posteriorly directed spines arranged in groups of from four to twelve or more, arising from a basal ridge. No sensorial organs were detected in the thoracic segments, but each of the abdominal segments bears, in addition to the spinose papillae, a pair of stout, elongate sensorial bristles, situated one on each side about the middle of the ventro-lateral aspect; each of these bristles is inserted on a short cylindrical base. The structure of segment XI (Figs. 6, 7) is complex and very different from that of the anterior segments; it is divided by a well-marked constriction into two distinct parts of about the same length, the anterior half being, however, subspherical in form, while the posterior half is subcylindrical, truncate posteriorly and distinctly narrower than the anterior half. The cuticular armature of the two parts is also very different; the anterior portion bears dorsally, laterally, and ventrally the spinose papillae characteristic of the rest of the body; the posterior portion bears dorsally and laterally a large number of fairly closely set, narrow, transverse highly chitinised bars, arranged in from twelve to fifteen irregular transverse rows. On the dorsal side the area bearing these bands is prolonged anteriorly for some little distance beyond the lateral level of the constrictions separating the two regions; and each side of this prolongation and, therefore, in the middle of the dorsal region of the segment, are the *posterior spiracles*, each opening at the end of a stout, cylindrical papilla. The segment bears a number of sensoria, the form and disposition of which is shown in the figures. On the dorsal side of the anterior part of the segment there is, on each side, near the antero-lateral angle, an elongate seta rising from a circular ring and apparently directed vertically; on the dorsal side of the posterior part, slightly in front of the middle of the region, is a row of four sensoria, of which the two outer elements are bristles, upwardly and backwardly directed, and the two inner elements, ring-shaped or short conical sensoria. On each side of the anterior portion, a little in front of the constriction separating the two regions, is a stout, elongate bristle, laterally directed; on the ventral side of the posterior part of the segment, and close to the anterior margin of this region, are four groups of what appear to be circular sensoria, each part being composed of a large central element and two small lateral elements, the two anterior groups being very close together while the

posterior groups are some little distance apart. The median part of the ventral surface of the posterior region is bare, but slightly behind the middle of this area there is inserted a pair of heavy, sharp-pointed bristles, backwardly directed: opposite to these, near the lateral border of the segment, there is on each side another similar bristle; neither of these bristles extends, in the specimens examined, quite to the level of the posterior extremity; no other sensoria, such as exist in the larvae of the Echinomyiine Tachinids, where they have a definite value as diagnostic characters, can be detected in this stage. The *tracheal system* in this stage is metapneustic. The *posterior spiracles*, which have already been described, are well developed and their position in the middle of the dorsal surface constitutes a very unusual anatomical feature. The felt chambers are so feebly chitinised that it is very difficult to determine their exact limits; they appear, however, to be unusually slender and elongate.

The *buccopharyngeal armature* (length 0·082 mm.) is of a very unusual type (Figs. 1, 3); it is quite different from that of the great majority of Tachinid larvae, the plan on which it is constructed being somewhat like that found in the primary larvae of *Sarcophaga* (Thompson, 1921), from which it differs, however, in the proportions of the various parts. Instead of a single median tooth, as in the majority of the first-stage Tachinids, there are here two lateral teeth, which are, however, so closely appressed that they can be seen only when the mouth-hook is examined from the dorsal or ventral aspect. The sclerites of the *intermediate region* are unusually elongate. The lateral *anterior sclerites*, and the *sclerite of the salivary canal*, are absent. The teeth of the *median anterior sclerites* are dark in colour, but the remainder of the organ is pale brown and only feebly chitinised. Each sclerite, in lateral view, is in form quadrangular, the posterior side being, however, somewhat narrower than the anterior side. The dorsal side presents a slightly irregular concavity: the ventro-anterior angle is slightly produced into a blunt downwardly directed tooth; from the base of this tooth, on the anterior margin, there arises a slender curved strip of chitin, directed forward and downward, and which is apparently connected by a cuticular bridge with a similar strip on the opposite side. Above the point of origin of this strip is inserted a fan-shaped group of three prominent teeth; the ventral element in each group is broad and leaf-like in form, the dorsal and intermediate elements much narrower, with moderately acute tips. The upper edge of the dorsal element is some little distance from the edge of the organ, so that the fan of teeth appears to arise from the side of its dorsal half. Seen in dorsal view, the two sclerites are slightly concave, touching or fused on the anterior half, where they are connected by a dorsal bridge of chitin, arising just behind the dorsal tooth of each group, diverging anteriorly and posteriorly, each sclerite being supported posteriorly by the anterior end of one of the sclerites of the intermediate region. The sclerites of the *intermediate region* are very elongate and slender, being about eight times as long as their dorso-ventral width at the middle. They are fairly heavily chitinised on the dorsal half, the ventral half being very pale in colour. A well-marked notch, roughly

rhomboidal in form, directed upward and backward, separates the posterior end of the lower half of the intermediate sclerite from the anterior end of the ventral wing of the basal sclerite, and indicates the point of entrance of the salivary canal. At their anterior ends, the intermediate sclerites are connected by a short bridge of chitin, bearing a pair of large, pale, oval, or circular spots, which probably contain sense organs. This bridge is probably homologous with the *epipharyngeal plate.* The *basal region* is well developed, remaining throughout the first stage unchanged in form; the upper border of the ventral wing is very slightly concave; the interalar space is rounded anteriorly, open behind with slightly diverging borders, the *dorsal wing* almost parallel with the *ventral wing*, but slightly longer and more slender, its posterior end rounded; the stem of the dorsal wing is broad, about one and one-third times as wide as the height of the intermediate sclerite at its middle; in front of the stem of the dorsal wing is a pale area bearing a number of interwoven chitinous threads; this area blends with the posterior part of the stem, which often exhibits a few pale, spindle-shaped spots; the fenestrated effect produced by the thread-like chitinous thickenings is very characteristic of this species; the dorsal wing at its middle is about one-fourth the width of the ventral wing.

Stage II. Dimensions: length 3–4 mm., width at widest portion about 0·25–0·5 mm., the larva grows very considerably during this stage, but no figures for the minimum and maximum dimensions have been obtained (Figs. 11, 13).

Cuticle thin and transparent, body fluids practically colourless; the tracheal system, the brown mid-intestine, and the sulphur-yellow Malpighian tubes clearly visible through the skin. A few small, inconspicuous, circular sensorial organs exist, but no setae or spines are present except in the last two segments, which bear a number of scattered spines, arranged in irregular groups and rows; these are acute but feebly chitinised and anteriorly directed; the last segment also bears a few rod-shaped sensoria, feebly chitinised and inconspicuous. The *head* is of the usual form, bearing in its dorso-lateral aspect the usual antennary and maxillary sensoria; the *antennae* are broadly convex in outline and somewhat wider than high; the maxillary sensoria are situated on a low conical protuberance lying ventro-lateral of the antenna; the respiratory system is, in this stage, metapneustic; the rudiment of the *anterior spiracle* can be seen on each side in the dorso-lateral region, a short distance cephalad of the caudal border of segment I; but its felt chamber appears to be solid and contains no gas. The *posterior spiracles*, unlike those of stage I, open on the posterior surface of the last segment; each is situated at the end of a short cylindrical protuberance; the felt chambers are unusually long and slender (Fig. 8) (length 0·108 mm.); each ends in a pair of small circular respiratory papillae; the distance separating the stigmata is, in this stage, about five times as great as the width of one of them at its base.

The *buccopharyngeal armature* (total length 0·154 mm.) (Fig. 15) is very different from that of the first stage, the *intermediate sclerites* being very short

as compared with the *basal sclerites*, while distinctly paired *mandibular hooks* are now present; the anterior hooks are completely separated from the intermediate region, which is itself fused with the basal region. The *anterior* or *mandibular hooks* consist of a pair of parallel, moderately elongate, slightly curved teeth, subacute at their tips, and arising from the dorso-anterior angle of a triangular basal portion, the apex of which is directed downward and slightly forward, constituting a ventral apophysis, subacute at its tip, to which attaches the tendon of the *mandibular depressor muscle*; these two apophyses diverge rather markedly, but on the dorsal edge the mandibles lie close together and are connected by a bridge arising just behind the base of the anterior tooth; the dorso-posterior angle of the anterior region is acute and terminates opposite the dorso-anterior angle of the *intermediate region*, which is short, the lateral sclerites being united to their anterior ends by the prominent *sclerite of the salivary duct*, their dorso-anterior angles projecting very slightly upward and outward as a short subacute tooth; the notch behind the sclerite of the salivary duct is rather deep and approximately quadrangular in form; the *basal region* is well developed, but distinctly shorter than the ventral wing and rounded at its tip.

Stage III (Fig. 17). The surface of the *cuticle* is fairly thickly studded with minute subacute transparent spines; on the first segment these are directed backward and are arranged in bands, which are most distinct near the anterior margin of the segment; on the last segment they are also arranged in bands but here are directed forward; on the remainder of the body no definite arrangement exists.

The *antenna* is inconspicuous, transparent, and only slightly convex; the *buccopharyngeal armature* (length 0·42 mm.) has now two articulations (Fig. 18); the *mandibular sclerites* are roughly triangular in form; the apex, which is ventrally directed, constitutes the *ventral apophysis* which receives the tendon of the mandibular depressor muscle; the anterior tooth is moderately stout, about the same length as the ventral apophysis, directed forward and slightly downward and acute at its tip; the dorso-posterior angle is definitely shorter than the ventral apophysis and truncate at its tip; between the dorsal edges of the mandibular sclerites, in some specimens, there extends a rather irregular transverse band of pigmented cuticle constituting a bridge, but this is not always present; a transverse ventral band lying below and in front of the hypopharyngeal sclerite also exists; the *intermediate sclerite*, seen from the side, is roughly T-shaped. The vertical arm, which is constituted by the sclerite of the *salivary duct*, slightly constricted at its middle, the anterior branch of the horizontal arm short, broad and rounded at its tip, the posterior branch about twice as long, slender and tapering to its tip; between the ventral apophyses of the mandibular sclerites and the sclerite of the salivary duct lies the semi-oval, heavily pigmented *hypopharyngeal sclerite* (Fig. 19), with a pair of large, pale, oval spots, containing sensorial organs; dorsad of this is the much smaller *epipharyngeal sclerite*, irregularly oval in form and also bearing sensorial

organs. There is a good deal of variation in the precise form of the parts of the buccopharyngeal armature, and a careful study of all the characters, combined with those of the preceding stages, is necessary before the larvae of some of the species of this group can be definitely determined. The *basal region* is well developed, the dorsal wing directed backward and upward, on the proximal half, rather markedly downward and backward on the distal half, a little constricted basally, expanding beyond the middle, tapering rather markedly toward its tip, the *ventral wing* about the same length as the *dorsal wing*, rounded posteriorly, the ventro-anterior angle of the basal region moderately prolonged anteriorly.

The *tracheal system* is metapneustic; the *anterior spiracles* (Fig. 4) (length 0·252 mm.) bear about fifteen to eighteen respiratory papillae, scattered over the surface and not restricted to the border of the felt chambers; the felt chamber is rather irregular in form and measures approximately 129 μ in length and 43 μ in width; the *posterior spiracles*, which are situated at the extreme end of the narrow, cylindrical last segment, lie in a pair of conical protuberances which harden, in the puparium, to form the prominent posterior stigmata; the felt chambers are short and broad, almost globular in form; each bears three short, oval spiracular slits (Fig. 9), of which the central element is laterally directed, while the other two lie respectively above and below it; the diameter of the tracheae is large, greatly exceeding that of the preceding stage.

Puparium: length 5–6 mm., width 2 mm. Formed within the skin of the dead host; colour, light reddish brown; the intervals between the segments often distinctly marked. Form, obconical, the anterior end rather broadly rounded, tapering to the posterior end, which is pointed, with a protruding cylindrical terminal portion, bearing the posterior spiracles; *anterior spiracles* small, black, moderately elongate; *posterior spiracles* separated by a shallow fossa, only slightly protuberant, each bearing three short, straight respiratory slits, the median slit lying alongside the horizontal diameter of the puparium, the other two above and below it, almost parallel to the dorso-ventral diameter of the puparium; *pupal respiratory apparatus* without the prothoracic cornicles, comprising only the *internal spiracle*, which is flat (diameter 0·144 mm.), circular in form, and bears seven or eight branching respiratory areas, provided with about a hundred rather large round spiracular papillae (Fig. 16).

Internal anatomy. The larva of *Plesina maculata* is extremely thin-skinned and transparent, so that in the earlier part of the second stage, before the adipose tissue has become filled with fat globules, it is possible to make out practically the whole of the internal anatomy by studying the living larva.

The *hypodermis* is thin and delicate, the hypodermal cells small and very numerous, forming a pavement epithelium of which the basement membrane is practically a plane surface; their small size seems to indicate that they increase by division as the larva grows, but so far no definite mitotic figures have been found.

The *muscular system* is composed of fibres which are unusually delicate and

feeble. When removed from the host and placed in saline, the larva contracts and extends rather feebly, but is apparently incapable of any real ambulatory movements. The integumental musculature is arranged on the same general plan as in *Miltogramma*, *Sarcophaga*, and *Digonochaeta*. Three groups of straight or *recti* muscles are present: a *dorsal* group composed of three fibres, a *ventral* group composed of four fibres, and midway between these a single *pleural* fibre. Across the middle of the pleural area, along the line of the circumference of the segment, there extends a band of *circular fibres*, usually composed of three elements, of which the posterior is markedly broader than the others; this band extends from the pleural border of the dorsal recti group to a point slightly ventral of the pleural border of the ventral recti group. In the integumental conjunctiva, extending from the pleural border of the dorsal recti group to about the middle of the ventral recti group, is the *intersegmental circular muscle*. In addition to these muscles, there are numerous *oblique muscles*, which, however, in spite of their rather complex and mechanically incomprehensible arrangement, are almost exactly similar in number and position to those of such free-living and semi-parasitic larvae as *Sarcophaga* or *Miltogramma*. One muscle of the dorsal recti group, present in *Miltogramma*, is absent here, as is also a small oblique muscle (No. 7, Thompson, 1928) lying between the dorsal recti group and the pleural rectus muscle; otherwise, all the muscles present in the *Miltogramma* larva are quite clearly recognisable in the larva of *Plesina*. The muscles of the ventro-pleural and ventral regions are also very similar, the only differences so far noted being that the oblique muscle, No. 18 (Thompson, *loc. cit.*), seems to be simple instead of trifurcate, and that one of the groups of oblique muscles 23–25, and one of the pair 19–20 are absent, just as in the larva of *Digonochaeta setipennis*.

The disposition of the muscles in the anterior and posterior parts of the body is very difficult to make out, because these segments are very slender and taper rather abruptly, and cannot be flattened after dissection without some distortion or destruction of the muscles. However, the general arrangement of the musculature of these regions is undoubtedly very similar to that of *Miltogramma* and *Sarcophaga*. In each of segments II and III there are in the mid-dorsal line a pair of small *cruciate muscles*, attached anteriorly to the border of the segment, crossing on the mid-dorsal line, and attached posteriorly to about the middle of the posterior half of the segment, just as in *Miltogramma* and *Digonochaeta*. In segment X there arise on each side two pairs of muscles which attach to the hind-intestine on a level with the middle of the segment: one member of each pair arises at the anterior end of the great pleural rectus muscle, the other at the anterior end of the ventral group of recti muscles; they meet at the same level on the intestinal wall around which they appear to form a ring. The penultimate segment is very similar to that in the other species mentioned, the only striking difference being the absence between X and XI of the circular intersegmental muscle and the presence of the muscles attached to the intestine; the musculature of the last segment differs markedly from that

of the rest of the body; it appears to be fairly similar to that of *Miltogramma*, but no attempt has been made to study it in detail.

From the anterior border of segment I to the posterior border of segment III, where they are inserted just in front of the imaginal disc of the haltere, there runs, on each side of the thorax, a group of *retractor muscles*, comprising several fibres; whereas in *Miltogramma* two sets are present, one situated ventrally extending from the anterior border of segment I to the posterior border of segment IV, the other situated ventro-pleurally and lying in the first three segments only.

The *buccopharyngeal muscles* are similar in arrangement to those of *Miltogramma*; between the mandibular sclerites are inserted the *elevators of the labrum*, each of which terminates in the dorso-lateral region of segment I; between these there runs forward a pair of broad, flat muscles, the *posterior dorsal protractors*, terminating in the mid-dorsal line not far from the anterior margin of segment I and a little distance in front of the elevators of the labrum; they are attached posteriorly to the side of the pharyngeal mass opposite the point where the oesophagus arises from it. Just above them are inserted the *pharyngeal depressors*, which attach on either side of the mid-dorsal line not far from the posterior border of segment I; ventrad of the pharyngeal depressors and dorsal protractors, there arise from each side of the pharyngeal mass, the *ventral protractors*, which are attached to the hypodermis in the middle of the ventro-pleural region of segment I; on each side of the mid-dorsal line, beneath the posterior end of the most dorsal fibre of the dorsal recti group of segment I, is attached the *dorsal pharyngeal retractor*, which is apparently inserted on the dorso-anterior angle of the intermediate sclerite; on either side of the ventro-lateral region of the pharyngeal mass, attached posteriorly to the pleural aspect of the posterior end of the pharynx, are the *depressor muscles of the mandible*, which attach anteriorly to the great ventral process of the paired hooks by the intermediary of a stout cuticular tendon. The *elevators of the mandible*, which are inserted posteriorly on the side of the pharyngeal mass, together with the depressors, are prolonged anteriorly in a tendon which is attached to the back of the dorsal protuberance just behind the base of the anterior hook of the mandible. The *retractors of the labium* and the *muscles of the salivary gland* have not yet been found.

The tracheal system is in this stage metapneustic. Between the *posterior spiracle*, on the end of segment XI, and the *rudiment of the anterior spiracle*, which lies in the dorso-pleural region near the posterior border of segment I, there runs on each side the *dorso-lateral tracheal trunk*, which in this species is unusually slender. Around the posterior part of the felt chambers of the posterior spiracles are the usual peristigmatic glands, containing numerous highly refractile droplets of secretion; a rather stout *posterior commissure*, which arises some distance in front of the anterior extremities of the felt chambers, connects the tracheal trunks in segment XI; some distance behind the anterior end of the trunks is the *anterior commissure*, which is slender and

extends forward and upward to the mid-dorsal line, which it reaches not far from the anterior border of segment II. Between these are *eight* additional *dorsal commissures*; the first of these arises in segment II, while the last is situated in segment VIII, so that their arrangement is not, at least in this stage, in strict accordance with the segmentation; *nine ventral branches*, of which the first eight are connected by *longitudinal commissures*, are also given off; these commissures, from which the trunks of the *rudimentary spiracles* arise, constitute the *ventro-lateral tracheal* trunks (Snodgrass, 1924) which become functional in the adult stage, the larval spiracles being developments of the dorso-lateral trunks and having no relation to the spiracles in the adult fly. In the mid-ventral line, at the junction of segments I and II, are *two ventral tracheal commissures*, which arise from the same ventral branch of the lateral tracheal trunks; one, whose apex is directed posteriorly, is M-shaped, the lateral angles of the M being anteriorly directed, and lying on a level with the posterior edges of the great ventral muscles of the pharyngeal mass; the central angle of the M is posteriorly directed, and terminates in the mid-ventral line, on the intersegmental conjunctiva between segments I and II; the posterior commissure is V-shaped, its apex, which is anteriorly directed, lying a short distance behind the intersegmental conjunction between segments I and II; between the matrices of these two commissures, which lie between the muscles and the hypodermis, is a protoplasmic bridge. The *tracheoles* are simple, not moniliform.

The *imaginal discs* are similar in arrangement and are similar in form to those of *Miltogramma*: in the stage III larva, *those of the first pair of legs* lie in the ventro-pleural region across the border separating segments I and II and are attached anteriorly to the hypodermis on either side of the mid-ventral line on a level with the points of insertion of the ventral protractors of the buccopharyngeal mass; *those of the second pair of legs* lie in the ventro-lateral part of segment II, extending posteriorly into segment III, and are attached anteriorly to the hypodermis about the middle of the segment, a little distance on either side of the mid-ventral line; *the discs of the third pair of legs* lie in the pleural region of segment III, near the posterior border, their apices attaching in the middle of the segment in the ventro-pleural region; *the discs of the wings* lie in segment III, above and in front of the disc of the third pair of legs, just inside the pleural fibre of the cephalic retractor muscles, the apex extending anteriorly over the margin of the segment and attaching to the hypodermis in segment II; *the discs of the halteres*, which are a good deal smaller than those of the wings, lie in the pleural region behind the anterior border of segment IV, at the anterior end of the great pleural rectus muscle; the stalk of this disc passes downward and forward between the hypodermis and the disc of the meta-thoracic leg, to its point of attachment. The other imaginal discs have not been studied.

The *fat body* is inconspicuous at the beginning of the hibernation period, but becomes opaque as the season advances, owing to the accumulation of fat

globules; the cells are small and closely united, forming plates in which it is impossible to distinguish the individual elements without staining. It comprises a pair of elongate, lateral bands, extending along the sides of the body cavity from the anterior part of segment X, where they seem to meet beneath the hind-intestine, to the anterior border of segment III, where they meet in the mid-dorsal line above the cerebroid ganglia; in some specimens there is a separate pair of anterior lobes lying in the mid-dorsal region above the area between the posterior end of the pharyngeal mass and the anterior end of the nerve cord; in segments VI and VII there is a pair of narrow lobes situated close together in the mid-dorsal region; a rather elongate, narrow band of adipose tissue connects the tips of the salivary glands. Finally, on the ventral side of segment V, is a pair of small plates of fat cells, which overlap in the median line, but are sometimes fused so as to form a single median pad.

The *nervous system* is of the usual condensed type found in the Cyclorrhaphous larvae and consists essentially in a pair of cerebroid or *supra-oesophageal ganglia*, fused in the median line and located in stage II in the third segment, and a *suboesophageal mass*, connected by lateral commissures to the cerebroid ganglia and extending posteriorly almost to the level of the posterior border of segment V. *Chordotonal organs*, segmentally arranged, are present, but no attempt has been made to study them in detail.

The *alimentary canal* opens anteriorly on the ventral side of the head, beneath the mouth-hooks, behind which is the *suctorial pharynx*, of which the supporting skeleton has already been described. This opens into the *oesophagus*, which is straight, slender, and unusually elongate, and extends backward, passing through between the cerebroid ganglia and the ventral nerve cord in segment III, and opening on a level with the conjunctiva between segments IV and V into the *oesophageal valve*, which is rather feebly differentiated; a short distance behind the posterior end of the pharynx there arises, on the dorso-lateral aspect of the oesophagus, slightly to the left of the median line, a well-marked cylindrical *caecum* (Fig. 36) with a constricted neck, slightly dilated toward its dorsal end, directed upward and backward, which appears to possess a thick, markedly convoluted chitinous intima, and contains a number of small, highly *refractile granules*, of which others, similar in form, occur in the oesophagus behind the point where the caecum is inserted; the opening of the caecum appears to be provided with a flap-like *valve*, directed backward; this organ seems to be homologous with the one found in the larvae of the Sarcophagine and Miltogrammine groups, and serves in *Miltogramma*, as the writer has shown, as a reservoir of the larval food, occupying, toward the end of the larval life, practically the whole of the body cavity. Behind the small oesophageal valve is the thin-walled *mid-intestine*, which is without any trace of gastric caeca; it runs backward as a straight tube, having about the same diameter as the oesophageal valve, into segment VII, where it opens into a voluminous *stomach*, extending anteriorly into segment IV, where it turns backward, diminishing rather rapidly in diameter and runs as a rather slender

tube into segment VIII, where it dilates again, the terminal portion being, however, rather slender; behind the distal dilation it passes into the *hind-intestine*, which is slender and terminates in the middle of the ventral surface of the last segment; the stomach and posterior part of the mid-intestine are almost always brown in colour, but the rest of the digestive tract is almost colourless. Immediately behind the mid-intestine are attached the *Malpighian tubes*, of which there are two pairs, both pale greenish yellow in colour, each pair opening into a common duct; the anterior pair runs forward into segment V, where it bends abruptly and runs backward, terminating in segment VII; the recurrent portion of the anterior tubes is thin-walled, dilated and filled with a dense, finely granular deposit, which is probably calcium carbonate; the posterior Malpighian tubes are much shorter than the anterior pair and follow a convoluted course in segment VII. The *salivary glands* are moderately well developed, composed of large cells with voluminous nuclei; they extend from the level of the posterior margin of segment III to the anterior border of segment I, where each opens into a slender duct with the usual tracheal-like intima, fusing with its fellow of the opposite side about the middle of the segment, the common canal opening above the sclerite of the salivary duct, already described.

The *circulatory system* is of the same type as that described by Pantel (1898) in the larva of *Thrixion halidayanum*; the *posterior region* or *ventricle* is provided with three pairs of *lateral valves*; it terminates posteriorly in the mid-dorsal line, not far from the posterior border of segment X; the posterior chamber, seen in lateral view and in optical section, is triangular in form and attached to the body wall posteriorly and dorsally by a number of fine fibre-like muscles; the three pairs of valves all lie in segment X, just as in the larva of *Miltogramma punctatum*, already described by the writer; the chamber in front of the anterior pair of valves extends forward into segment IX, passing beneath the dorsal tracheal commissure of that segment; in front of this point its diameter diminishes and the vessel takes on a vacuolated appearance, due to the development of the *proventricular cushion*, described by Pantel in *Thrixion* and considered by him to have a valvular function; in this species the proventricular cushion is about three-fourths as long as the first chamber of the ventricle; on each side of the ventricle are two *suspensory muscles* whose bifurcating fibres are attached medially to the sides of the ventricle, above and below the pericardial cells, and laterally appear to be attached to the lateral tracheal trunk, which moves regularly in time with the beats of the heart; on each side of the ventricle, extending from below the second chamber almost to the proventricular pad, is a row of five or six large *pericardial cells* which lie close to the ventro-lateral surface of the heart; from the proventricular cushion the dorsal vessel extends forward to the mid-dorsal line, terminating in the posterior part of the pharyngeal mass; in segments VI–VIII it lies ventrad on one of the dorsal lobes of the fat body; other details can only be made out from sections.

In a stage II larva, examined in saline, a short time after it had been extracted from the host, and had its posterior spiracles in contact with the air, there were about 120 heart beats to the minute.

(2) *Melanophora roralis* L.

Stage I. The larva is elongate (length 0·42 mm.) and slender.

The general form of the *buccopharyngeal armature* (Fig. 38) (length 0·086 mm.) is like that of *Plesina maculata*. The *anterior sclerites* are closely appressed, though the two sets of teeth are well separated and distinct; the sclerites are roughly trapezoidal in form, with the small extremity of the trapezium posteriorly directed, the postero-dorsal angle acute, the postero-ventral angle obtuse, the antero-ventral angle truncated, the antero-dorsal angle somewhat rounded, the dorsal side of the sclerite presenting a very broad, shallow, angular notch; the anterior teeth, which form a group whose base is about equal to half the height of the anterior side of the sclerite, appear, when seen from the inside, to comprise a dorsal bifurcate element, formed of an acute dorsal part projecting anteriorly considerably beyond the other teeth, rounded above, straight below, and an acute ventral part directed somewhat ventro-anteriorly; and a rather broad leaf-shaped ventral element. When examined from the outside, two parts of the dorsal element are seen to be separated by a short dark suture, another similar suture lying between the low part of the bifurcate element and the leaf-shaped ventral tooth. A pale, somewhat crescent-shaped area, having its convexity anteriorly directed, is sometimes visible on the base of the group of teeth. The *intermediate region* is long and narrow, with the dorsal half dark brown and chitinised, the ventral half pale in colour and soft, except on the anterior half, where there is a narrow brown strip extending backward for some distance where it gradually becomes indistinct. On the dorsal side of the anterior end of the intermediate sclerite is a small circular sensorial organ. The opening of the *duct of the salivary gland* is well marked, but no distinct *salivary sclerite* exists; the dorsal wing of the *basal region* is dark brown, with an irregular narrow paler band near the dorso-anterior border, the apex of the dorsal wing is more or less truncate; along the dorsal edge of the ventral wing, which is straight, runs a narrow, dark brown band contrasting sharply with the pale coloration of the remainder of this wing; ventral wing about twice as wide as the dorsal wing.

The *antennae* (Fig. 42) are subcylindrical, elongate (length 0·0096 mm.), about two and a half times as long as wide at base, with the tip subacute; the *skin* bears numerous small scales, pointed, rather broad at the base, but quite feebly chitinised and pale in colour; on the pleural areas are some very broad, narrowly overlapping scales; the conical sensoria are also present on most of the segments, but on the last segment, which is tapering, there are a number of sharp, stout, elongate setae: two pairs on the dorsal side in front of the posterior spiracles, one pair on the dorsum, just behind the spiracles, one on each side on a level with the spiracles, and two pairs of curved stiff spines inserted behind

the level of the spiracles and directed posteriorly. The posterior tracheae open, as in *Plesina*, in a pair of divergent, elongate cylindrical *stigmata*, each about twice as long as wide, situated on the middle of the dorsal side of the last segment. The only complete specimen of this stage on hand is, however, so undeveloped that an accurate detailed description is not possible.

Stage II. Very few specimens of this stage have been discovered in the dissections. The larva is rather smaller than such species as *Plesina maculata*, rather slender, with transparent, unchitinised, and practically unarmed cuticle; the *antennae* are short and only slightly convex: the *tracheal system* metapneustic with small posterior spiracles, provided with slender, inconspicuous *felt chambers*; the *buccopharyngeal armature* (Fig. 44) (length 0·1107 mm.) is very similar in form to that of *Plesina maculata*, from which it can be distinguished only with difficulty; *anterior sclerite* moderately elongate, slender, slightly curved, somewhat longer than the downwardly directed process forming the basal part of the sclerite; *intermediate region* acute anteriorly, its upper edge oblique, and with only a slight shallow depression before the dorso-anterior angle of the dorsal wing of the basal sclerite, into which it passes almost without a break; anterior half of the intermediate region straight on its ventral border, the posterior half constituted by the rather prominent *sclerite of the salivary gland*; dorsal wing of the *basal region* directed upward and backward, ventral wing backward and slightly downward, the interalar notch triangular, rather markedly acute anteriorly; the armature is rather heavily pigmented with a pale line a little below the upper edge of the intermediate region; the dorsal and ventral wings of the basal region also tend to become paler distally.

Stage III. The *buccopharyngeal armature* (Fig. 40) (length 0·336 mm.) of this stage is very easily recognised, since it differs from those of all the other members of the group so far studied in the absence of an articulation between the basal and intermediate regions.

The *anterior* or *mandibular sclerites*, are rather feebly developed though heavily pigmented, approximately triangular in form, with a rather elongate, slender, somewhat irregular ventral apophysis, an obliquely truncate postero-dorsal angle, and an anterior tooth which is short, slender, directed anteriorly, but curving slightly downward toward its apex, and acute at the tip; the *intermediate region* is fused with the *basal region*; its antero-dorsal angle is produced as a short obtuse tooth, articulating with the postero-dorsal angle of the anterior sclerite; just behind the anterior border of the ventral side is a deep oval notch, in front of which is the bridge-like *sclerite of the salivary duct*, fused at the sides with the intermediate sclerites; the *basal regions* are moderately large, though rather feebly pigmented; the triangular region between the base of the dorsal wing and the opening of the salivary duct is unusually elongate; the *dorsal wing* is somewhat scimitar-shaped, narrow on its basal half, but expanding distally, although acute at tip; the *ventral wing* is sometimes hardly more than half the length of the dorsal wing, though in other

specimens it is about the same length; it is obliquely truncate at tip; in the interval between the ventral apophysis of the mandible and the sclerite of the salivary duct is the large *hypopharyngeal plate*, semi-oval in form, with two large oval colourless spots, probably bearing sensorial organs.

The *tracheal system* is metapneustic; the *anterior spiracles* (Fig. 41) (length 0·12 mm.) with the extremity flattened and bearing half a dozen spherical respiratory papillae. The *posterior stigmata* (Fig. 43) are moderately protuberant, each bearing three straight respiratory slits.

Puparium: length about 4·5 mm., width about 1·3 mm. Colour, pale yellow, almost translucent, subshining, cylindrical, the anterior end rounded, with slender but rather prominent and slightly divergent stigmata, the posterior end (Fig. 39) terminating in a conical protuberance, bearing the small stigmata, separated by a slight depression. The *internal spiracle* has relatively few, large-sized papillae (Fig. 45).

Internal anatomy. Very few larvae of this species have, so far, been found in dissection so that only the main features of the internal anatomy can be described.

The *alimentary canal* resembles, in its general characters, that of *Plesina*. Just behind the pharyngeal mass there arises the *caecum*, which is, as usual, on the left side; it is rather small in this species but contains the *refractile granules* present in other members of the group. The *oesophageal valve* lies in the posterior part of segment III. It is rather small; no *gastric caeca* are present. The dilated portion of the mid-gut or *stomach*, which lies in segments V–VII inclusive, is yellow in colour, as in *Plesina*, but rather more elongate and slender. The *posterior intestine* opens on the ventral side between segments X and XI. The *salivary glands* extend from the anterior border of segment II to the anterior part of segment IV, have the anterior third with a rather voluminous canal, while the remaining portion is subdivided by a number of slight constrictions into a series of spherical segments. The cytoplasm of its cells contains numerous minute refractile granules. The *Malpighian tubes* are of the usual type, the posterior pair, which are short and simple, being situated in segment VII, while the anterior pair are more elongate; the recurrent branches of the anterior glands, which lie in segments V and VI, are rather shorter than usual and filled with a dense mass of white deposit. The *hypodermal cells* are small and inconspicuous; the *circulatory system* is of the same type as is found in *Plesina*; the *heart*, which lies in segments VIII and IX, presents no unusual features; but the *pericardial cells*, though fairly large, are, in this species, colourless and inconspicuous.

The *muscular system*, so far as could be determined, is practically identical with that of *Plesina*, at least in the anterior abdominal sections; as in the related forms, the fibres are rather weak and slender.

The *tracheal system* resembles that of *Plesina*; it is metapneustic; the posterior tracheal commissure is situated in segment X. The inconspicuous *rudiment of the anterior spiracle* lies near the posterior edge of segment I in the

dorso-pleural region; the *peristigmatic glands* are relatively inconspicuous; the posterior *felt chambers* are clearly more slender than the tracheal trunks immediately in front of them; the *nervous system* presents no unusual features; the *cerebroid ganglia* are situated in the middle of segment III, while the ventral nerve cord extends backward into segment V. The *fat body* comprises two small dorsal lobes lying in segment II and a broad band on each side of the body extending from the anterior border of segment IV to the anterior part of segment X. From each of these bands an additional dorsal lobe, given off in segment IX, runs forward to the anterior end of segment IV; the cells of the fat body of *Melanophora roralis* are often filled with numerous small, highly refractile granules, so that this tissue appears opaque by transmitted light, but dead white by reflected light. The *gonads*, which are, as in *Plesina*, rather small in size, lie embedded in the fat body in segment VIII.

The conspicuous white colour of the adipose tissue, which is probably due to the accumulation of carbonates in the fat cells, is the most striking characteristic of this larva and at once distinguishes it from other members of the group, even in the second stage when the buccopharyngeal armature is very similar to that of *Plesina maculata*; but some specimens, believed to belong to this species, have not exhibited this feature.

(3) *Phyto melanocephala* Meig.

Stage I. No complete specimen of this stage has yet been found, the only structures available for description being the buccopharyngeal armature and the antennae (Fig. 25). Whenever any deeply pigmented cuticular structures, such as scales or spines, are present, these can practically always be seen on the skin attached to the mouth-hooks; here, nothing of the kind is visible, so it may be assumed that the cuticle is unarmed, or, at most, provided with very feebly pigmented scales or spines.

The *buccopharyngeal armature* (Figs. 27, 28) is of the *Plesina* type, with one articulation, the *anterior region* consisting of a pair of sclerites lying close together and connected by a dorsal bridge of chitin (length 0·1107 mm.). The dorsal third of the *intermediate region* is well pigmented and chitinised, forming a narrow elongate band, on the anterior extremity of which is a small circular sensorial organ; below the anterior part of the chitinised strip is a similar, but much shorter one, which may be compared to the hypopharyngeal plate in later stages. The *anterior region* is approximately trapezoidal with the small extremity directed posteriorly; a shallow indentation occurs both on the dorsal and ventral side, in front of the posterior border. What appears to be a chitinous bridge extends between the anterior halves of the dorsal edges of the anterior sclerites. The *teeth*, which are present in other species, form, here, a semi-oval plate, inserted on the dorsal half of the anterior margin of the sclerite. This plate, which under a low power appears to be simple, is entire on the dorsal edge, but presents, on the ventro-anterior margin, a series of about seven small teeth, separated by distinct, dark striations, extending backward on to the

body of the plate, the dorsal tooth being the most distinct and the most acute. The form of this plate is quite characteristic of the primary larvae of *Phyto melanocephala*. The dorsal wing of the *basal region* is broadly attached to the ventral wing, but the posterior part, which extends backward to a point some distance beyond the level of the top of the ventral wing, is slender, though rounded at its tip. The *ventral wing* is pale in colour, but the *dorsal wing* is rather heavily pigmented, except on the anterior border of the "stem," where it is somewhat paler and presents a few irregular fenestrations.

The *antennae* are elongate and slender (Fig. 25) (length 0·021 mm.), enlarging somewhat at the base.

Stage II. The *buccopharyngeal armature* (Fig. 31) (length 0·144 mm.) is of the *Plesina* type. The *anterior region* is composed of a triangular basal portion, of which the downwardly directed apex constitutes the ventral apophysis, receiving the tendon of the *depressor muscle of the mandible*; on its ventro-anterior border, just below the dorso-anterior angle, is the stout *mandibular tooth*, slightly curved toward its end and acute at the tip; the *anterior sclerites* are distinctly separated from the *intermediate region*, but this is fused with the *basal region*; the *intermediate region* is rather broad and elongate. The part in front of the *sclerite of the salivary duct* is about three-eighths of the length of the whole of the lateral sclerite from its tip to the notch into which the salivary duct opens; the *sclerite of the salivary duct* is not prominent, projecting only very slightly below the ventral border of the lateral sclerites with which it is fused; the notch of the salivary duct is distinct, the *basal region* is well developed, the *ventral wing* about two and a half times as long as the intermediate region, the *dorsal wing* broad at its origin with its dorsal edge rounded, its ventral apex straight, subacute at the tip; the dorsal and ventral wings are rather markedly divergent, the dorsal wing distinctly shorter than the ventral wing.

Stage III. The *buccopharyngeal armature* (Fig. 33) (length 0·504 mm.) resembles that of *Plesina maculata*; the *anterior* or *mandibular sclerites* are composed of a rather narrow basal portion prolonged anteriorly into a short, stout tooth, directed slightly downward and acute at tip, and posteriorly into a rather prominent apophysis, obliquely truncate at its end; the dorsal apophysis is rounded, but not very prominent; the ventral apophysis is unusually elongate, slightly more than three times as long as the anterior tooth; the *intermediate region* is only a little longer than the anterior region; the *sclerite of the salivary duct*, which is fused at its end with the lower border of the centre of the lateral sclerites, prominent and distinct, expanding toward the mid-ventral line; the lateral sclerites are slightly concave on the dorsal edge, with an anterior branch about twice as long as wide and rounded at its end and a posterior branch about one and a half times as long as the anterior branch, subacute or rounded at its end. Between the anterior and intermediate sclerites is a large *hypopharyngeal* and a smaller *epipharyngeal* sclerite, both chitinised and brown in colour, but bearing transparent spots which contain

sensorial organs; the *basal region* is well developed, the anterior border oblique, without any distinct angle or bend between it and the upper edge of the *dorsal wing*, which is hardly narrowed at its origin, but tapers toward the tip, which is subacute; the *ventral wing* is about the same length as the dorsal wing.

Puparium: length about 7 mm. Colour, light reddish brown, subcylindrical, but with the dorsal surface convex and the ventral surface rather flattened; at the posterior extremity the usual cylindrical protuberance, bearing the two small brown, shining stigmatic areas (Figs. 35, 37), each with three short, straight slits radiating from a common centre upward and outward; in the only specimen obtained up to the present, the puparium is rather markedly arcuate in lateral view, dorsal surface convex, the ventral surface concave; but this is probably due to the fact that it was formed in the body of an *Armadillidium*; papillae of the *internal spiracle* small (Fig. 34).

Internal anatomy. The internal anatomy is, in general, similar to that of *Plesina*; the *hypodermal cells*, near the anterior extremity, are sometimes convex, but are flattened toward the middle of the body; the *muscular system* resembles that of *Plesina*, but was not studied in detail owing to the lack of suitable material; the *alimentary canal* is of the *Plesina* type, with a dorsal *oesophageal caecum*, a rather inconspicuous *oesophageal valve*, no *gastric caeca*, a relatively short *mid-intestine*, of which the posterior part dilates to form a large brown or yellowish *stomach*, and a *posterior intestine* which narrows rather suddenly in segment VIII and opens to the exterior on the ventral surface at the junction between segments X and XI. The *salivary glands* extend from the middle of segment III to about the beginning of segment V, where they are joined by a short connecting lobe of adipose tissue; they are of the usual simple cylindrical type; the *Malpighian tubes* comprise a short posterior pair and an anterior pair of which the recurrent portion is dilated and filled with *carbonate* granules; the *nervous system* is of the typical form and extends from the anterior part of segment IV into segment V; the *circulatory system* resembles that of *Plesina*; the heart, which is situated in segments VIII and IX is bordered on its posterior three-fourths by seven or eight rather large *pericardial cells*, deep yellow in colour. The *tracheal system* is metapneustic; the rudiment of the *anterior spiracle* is situated in the dorso-pleural region between segments I and II; it is flat and not spherical as in certain other species of the group (*Frauenfeldia*); the *posterior tracheal commissure*, which is very short, lies in the middle of segment XI; the *posterior peristigmatic glands* are only moderately well developed; the *fat body* is of the usual type, consisting, as in *Plesina*, of a plate of small mononuclear cells; a broad ventro-lateral band extends on each side from the middle of segment II into the beginning of segment X; in segment IX this gives off a narrow dorso-pleural band extending forward to the middle of the stomach beside the mid-dorsal line; the cells of the fat body are, for the most part, semi-translucent without fat globules, and contain no deposits of urates or carbonates; the *gonads* lie embedded in the fat body in segment VIII.

(4) *Styloneuria discrepans* Pand.

Stage I. The first-stage moult skin, which of course carries with it the buccopharyngeal skeleton, very seldom remains attached to the stage II larva of *Styloneuria*. It probably works backward and passes out through the orifice of the respiratory sheath. On several occasions it has, however, been found. No pigmented scales or spines have been detected on the cuticle attached to it, so that the larva of this species, like those of *Melanophora* and *Phyto*, is probably colourless and relatively unarmoured.

The *buccopharyngeal armature* (Figs. 20, 24) (length 0·095 mm.) is of the *Plesina* type, and sharply characterised by the fact that the dorsal wing of the *basal sclerite* is absent, being replaced by a mere rounded or subtriangular projection. The *anterior region* is composed of a pair of closely appressed plates, approximately quadrangular in form, with concave sides; the ventro-anterior angle is somewhat prominent, truncate at the end, with the dorso-posterior angle produced backward as a rather prominent broad tooth, acute at its extremity. On the dorsal half of the anterior border is situated a group of three prominent subacute, curved teeth, well separated at their tips by short narrow notches, and posteriorly by dark striations. The *intermediate region* is elongate and narrow, the dorsal half light brown in colour, the ventral half pale, though on the anterior half the ventral portion of the sclerite is pigmented like the dorsal half, from which it is separated by a pale narrow line. The opening of the *salivary duct* is distinct but no *salivary sclerite* exists. The *basal region* consists of a short *ventral wing*, pale in colour and truncate posteriorly, and a subtriangular dorsal projection, apically rounded, and darker than the ventral wing. No *dorsal wing* has been found in any one of the several specimens examined from a number of different localities.

The *antennae* (length 0·0096 mm.), though elongate, are somewhat stouter than in some of the other species of the group, tapering to the tip, which is rounded.

Stage II. In this stage the larva is elongate and slender, with a thin transparent colourless cuticle, without any pigmented scales or spines, even on the last segment; to the absence of such spines is possibly due the fact that the first-stage moult skin is seldom attached to the body of the second-stage larva, whereas in *Plesina*, where some spines are present on the last segment, it usually remains as a narrow collar, around the posterior part of the body; the *respiratory system* is metapneustic; the *posterior spiracles* are situated on the last segment, each opening in a short rounded pigmented stigma. The felt chambers of these spiracles are moderately elongate and slender (length 0·045 mm.). The *antennae* in this stage are quite inconspicuous, short and hemispherical in form.

The *buccopharyngeal armature* (Fig. 23) (length 0·12 mm.) is similar in its general structure to that of *Plesina*, differing from it only in the proportions of the various parts. The *epipharyngeal plate*, lying in the dorsal pharyngeal wall, is small, roughly quadrangular in form and bearing about half a dozen small

pale spots, of which at least two bear sensorial organs; the *hypopharyngeal plate*, which lies in the ventral wall of the pharynx opposite to the one just mentioned, is considerably larger, with a pair of large oval pale spots, each of which contain a small punctiform sensorium. As in other species of the *Plesina* group, only one articulation, situated between the intermediate and anterior regions, is present in this stage. The *anterior sclerites* or *mandibles* consist of an approximately triangular basal portion, the apex of which almost touches the anterior end of the intermediate region, while its base lies across the side of the buccal opening; the ventral angle of the base receives, at its tip, the tendon of the *retractor of the mandible*, the dorsal angle receives the tendon of the *elevator of the mandible*; at this point, also, a chitinous bridge firmly joins the two *mandibular sclerites*, which lie close together dorsally, but diverge rather markedly ventrally; from the anterior side of the basal portion of the sclerite arises the curved, slender *tooth of the mandible*, which is directed forward and downward, and is acute at its tip. The *intermediate region* is rather elongate and slender, its length, measured from the posterior side of the notch into which the salivary gland opens, to the anterior end, being about four and a half times as long as its height at the anterior end; the *sclerite of the salivary gland*, which joins the *intermediate sclerites*, is not very distinctly marked off from the rest of the intermediate region when seen from the side, though when the armature is examined in dorsal view, it is seen to extend between almost the whole length of the lateral sclerites; the notch into which the salivary gland opens is short and shallow; the dorsal edge of the intermediate sclerite rises gradually toward its posterior end, so that it passes, without any abrupt transitions or angles, into the *dorsal wing of the basal sclerite*, which is rather broad and short and rounded posteriorly; the *ventral wing of the basal sclerite* is slightly longer than the dorsal wing; its posterior extremity is obliquely truncate, the ventral angle projecting somewhat beyond the dorsal angle; between the ventral wings, the ventral wall of the pharynx is heavily chitinised and pigmented, but smooth and without ridges.

Stage III. The *cuticle* is practically naked, but some areas of small acute spines, anteriorly directed, exist on the last segment; the *antenna* is small, inconspicuous, globular in form. The *buccopharyngeal armature* (Fig. 29) (length 0·384 mm.) is heavily pigmented and strongly chitinised; the *intermediate region* is separated from the anterior and basal sclerites by distinct articulations; the mandibular sclerites, which are distinctly separated, are rather small as compared with the rest of the armature; the *ventral apophysis* is elongate, being somewhat larger than the *mandibular tooth*, which is directed straight forward, rather short and stout, only slightly curved and moderately acute at the tip; above and behind the base of the tooth is the prominent rounded *dorsal apophysis*; posteriorly the mandibular sclerite is subtriangular in form, its tip articulating with the anterior end of the intermediate sclerite; the *intermediate sclerite* is triangular in form, its base, which is ventrally directed, being shorter than the other two sides; the apex of the sclerite is directed

upward and backward so as to lie along the anterior margin of the basal region, the ventro-anterior angle of which articulates with the notch between the posterior margin of the intermediate sclerite and the transverse *sclerite of the salivary gland*, which is heavily pigmented and forms a band joining the two intermediate sclerites; the *basal region* is large and heavily pigmented, the *dorsal wing*, which is acute at the tip, being slightly longer than the ventral wing; the stem of the dorsal wing is broad; a rather large pale area covers the dorsal two-thirds of the basal wing just behind its tip; in the notch between the ventral apophysis of the mandibular sclerite and the intermediate sclerite, there is a rather narrow, curved, heavily pigmented sclerite, running across the floor of the buccal cavity; the lateral apices of this sclerite lie along the ventral edge of the intermediate sclerite; a large, oblong *hypopharyngeal sclerite* bearing a pair of pale circular spots containing sensoria, lies on the ventral side of the pharyngeal cavity, in the space between the anterior ends of the intermediate sclerites and the sclerite of the salivary gland; above this, in the dorsal pharyngeal wall, is a smaller *epipharyngeal sclerite*, which also bears a pair of sensorial organs.

The tracheal system in this stage is *amphipneustic*; the *anterior spiracles* (Fig. 26) are large and projecting, measuring about 0·144 mm. in length from the base of the felt chamber to the tip; the apical part flat, distinctly longer than broad, and having 12–14 short, oval respiratory papillae regularly disposed along its edge, the inner and outer surfaces being bare, though in some specimens the arrangement is less regular, a partial row comprising several additional papillae, sometimes arising behind the row on the border of the felt chamber; the *posterior spiracles* are large and protuberant, with short voluminous felt chambers, about half as broad as they are long (see description of puparium); the tracheae are now enormous as compared to the preceding stage, their diameter in stage III being about fifteen times as great as in stage II.

Puparium: length 7 8 mm., width 3–4 mm. (Fig. 30), slightly flattened dorso-ventrally, having the ventral side, which corresponds to the ventral side of the dead body of the woodlouse, in which the parasite pupates, markedly less convex than the dorsal side; colour, reddish brown, subshining, the intersections between the segments well marked on the dorsum, elsewhere indistinct; form, seen from above, subcylindrical, the anterior end broadly rounded, the posterior end more acute with a subcylindrical posterior projection, bearing at its end the posterior spiracles, which are separated by a shallow fossa; *anterior spiracles* slender but prominent, *posterior spiracles* (Fig. 34) bearing three or four short straight slits radiating outward, upward, and downward, their arrangement being, however, rather variable; *pupal respiratory apparatus* without the prothoracic horns, comprising only the *internal spiracle*, which is rounded (diameter 0·276 mm.), bearing a larger number of rather small respiratory papillae arranged along irregular areas radiating from the centre of the spiracle (Fig. 22).

Internal anatomy. The internal anatomy is, in general, practically identical

with that of *Plesina*. The *alimentary canal* presents the usual *oesophageal caecum* in which, however, the refractile granules are not invariably present; the *oesophageal valve* is situated in segment IV and is, as in *Plesina*, rather inconspicuous; the *mid-intestine* possesses no gastric caeca; it is more or less conspicuously dilated in certain regions; the initial portion, which in one specimen examined contained groups of fat globules, some in the gastric cavity, some in the intestinal wall, extends backward into segment VI, where it passes to the dorsum and then forward into segment V, where it opens into a narrower section, which extends back into segment VI, down to the ventral surface and then forward, opening in the same segment in the large brown *stomach*, which extends through segments VI, VII, and VIII; the *hind-intestine* opens to the exterior on the anterior edge of the ventral side of segment XI. The *muscular system* is essentially similar to that of *Plesina*. The *tracheal system* is, as usual, metapneustic, with the *posterior transverse commissure* lying about the middle of segment X; the tracheae are very slender; the *rudimentary spiracles* were readily seen in certain specimens of this species examined shortly after removal from the host; it may be noted that the tracheoles running to the rudimentary spiracles sometimes, at least, arise, not from the ventral tracheal trunk, but from the dorso-ventral commissures, running between the dorsal and ventral trunks. The *salivary glands* are similar to those of *Plesina*, and extend from the anterior part of segment II to a point not far from the end of segment V. The *Malpighian tubes* are of the usual type; the recurrent section of the anterior tubes, which extend through segments V, VI, and VII, is filled with the usual deposit, presumably *calcium carbonate*. The *central nervous system* extends from the posterior third of segment III to near the posterior end of segment IV. The *fat body* is composed, as in *Plesina*, of plates of small cells and comprises principally a long lateral plate on each side of the body, with a dorsal anterior projection through segments II and III. The *circulatory system* is similar to that of *Plesina*; the *ventricle* extends posteriorly some little distance into segment X, and forward through about two-thirds of segment IX; it comprises the usual three chambers of which the anterior is partly closed by the *ventricular pad* of vacuolated cells already described in other species; six or seven large *pericardial cells* lie on either side of the ventricle. Groups of *oenocytes*, which are moderately large but thin, transparent, and rather inconspicuous, exist on the ventro-lateral region of some of the abdominal segments, in the space between the ventro-lateral and pleural recti muscles, between the circular intrasegmental muscle fibres and the body wall.

(*b*) Frauenfeldia *group*

(1) *Frauenfeldia rubricosa* Meig.

Stage I. No complete specimen of the primary larva of this species is as yet available; it is, however, evident from the fragments of the skin attached to the first-stage mouth-hooks, that the larva of *Frauenfeldia* possesses a well-

developed cuticular armature, formed by groups of rounded areas covered with a chitinised and pigmented plate, in the centre of which is a circular depression in which in turn is inserted a cylindrical structure from three to four times as long as wide, sometimes bearing a short, stiff bristle at its extremity (Fig. 67). These organs, which appear to be sensorial in function, are arranged along the circumference of the segments which bear them; but at present it is not possible to determine their number and position exactly.

The *antennae* (length 0·041 mm.) are elongate, slender, and whip-like (Fig. 63), tapering gradually from base to tip, without any thickening in the distal portion, inserted in a broad but low, chitinised and pigmented collar.

The *buccopharyngeal armature* (Figs. 51, 59) (length 0·192 mm.) very closely resembles that of *Cyrillia angustifrons*, being, however, much longer.

The armature is composed, as in *Cyrillia*, of an anterior sclerite and a posterior region, comprising intermediate and basal sclerites fused together.

The *anterior sclerite* (Fig. 59) resembles that of *Cyrillia* in its general form; it is composed of two closely appressed halves. Two ventral teeth are certainly present, but there are not, in my specimens, any clear indications that the dorsal tooth is paired. This sclerite consists of a heavily chitinised roughly quadrangular, basal portion; its posterior margin is convex; the dorso-posterior angle is rounded, the ventro-posterior angles seem to be connected by a flat, rather prominent bridge; the ventro-anterior angle seems to be acute, and directed anteriorly, but it is covered by a rather broad strip of chitin, which bends upward on either side of the sclerite so as to cover its ventro-posterior angles and may perhaps be fused with it at the ends; opposite the dorso-posterior angle and a short distance from it, is an unchitinised oval spot; anteriorly the anterior sclerite is prolonged into two large, acute, curved teeth, of which the dorsal one is two-thirds as long as the basal part of the sclerite; the ventral tooth is less than half the length of the dorsal tooth. The *intermediate region* is elongate, dark brown in colour, practically straight; it is about eleven times as long as its height at the base; the anterior end is truncate, the ventral angle a right angle, the dorsal angle rounded; the opening of the *salivary duct* is distinct though no separate sclerite is present; it is opposite the middle of the stem of the dorsal wing of the basal region; the *basal region* presents the usual dorsal and ventral wings, both distinct and well developed; the *dorsal wing* consists of a broad, short, heavily chitinised stem, whose dorso-anterior angle is distinct, while the dorso-posterior angle is prolonged backward to form a slightly curved dorsal wing; the *ventral wing* is less strongly pigmented than the dorsal wing, but of about the same length.

Stage II. The larva differs from those of the *Plesina* group in its rather markedly spindle-shaped form. The cuticle is a good deal thicker and tougher than in *Plesina*. No spines or scales appear to be present except in the last segment, where there are some scattered, short, brown spines, directed anteriorly and probably used to anchor the larva to the integumental sheath. The *posterior stigmata* are situated on the dorso-posterior aspect of the last segment;

they are short, cylindro-conical, and protuberant, sometimes brown in colour; each presents three small, oval, circular, stigmatic papillae in this stage.

The *antennae* are markedly elongate and slender (length approximately 13 μ) about five times as long as wide at the base.

The *buccopharyngeal armature* in this stage (Fig. 60) is similar in form to those of *Cyrillia angustifrons*, species A and species B, but differs markedly from those of all other second-stage larvae of this group (length, *in toto*, 0·18 mm.).

The *anterior hooks* or *mandibles*, which are distinctly separated and divergent at their tips, consist of a basal part, roughly triangular in lateral view, with the apex, which is truncate, directed backward, and the base, which is somewhat rounded, directed anteriorly, and prolonged into a slightly curved tooth, the length of which varies very considerably in specimens apparently belonging to the same species; behind this tooth is an ill-defined, pale spot, oval in form; below the ventro-anterior angle of the anterior sclerite and distinctly separated from it is a short narrow band of chitin, rather irregular in outline; the *intermediate sclerite* is distinctly separated from the *posterior sclerite* behind; it is elongate and narrow, about five times as long as its breadth at the widest part, and narrows considerably from the posterior to the anterior end, which is rounded; the ventral angle of the posterior end is rounded, while the dorsal angle is acute, the posterior end of the intermediate region being cut off obliquely so that this region is prolonged dorso-posteriorly into a point, which articulates with a notch in the anterior edge of the basal sclerite; above the anterior ends of the intermediate sclerites is a small oblong *epipharyngeal plate*, with a pair of small transparent spots containing sensoria, at its anterior end. A *hypopharyngeal plate* lies below the sclerites, opposite the other; a long ventral plate which projects posteriorly to receive the duct of the salivary gland joins the ventral edge of the intermediate sclerite on their posterior three-fourths. The *basal region* is of the typical form, with the usual dorsal and ventral wings connected by a broad bridge; the anterior border of the bridge is oblique, the ventral angle projecting forward as an acute tooth, a little below the dorso-posterior angle of the intermediate sclerite; a short distance above the point of the tooth is a shallow notch, with which the posterior end of the intermediate sclerite articulates; the *dorsal wing* is somewhat longer than the *ventral wing* and both are slightly irregular in form; the floor of the pharynx, between the ventral wings, is unpigmented. The larva in this stage is metapneustic, the *posterior stigmata* small, the felt chambers rather elongate and slender (length 0·084 mm.).

Stage III. The *buccopharyngeal armature* (Fig. 65) (length 0·420 mm.) is, in general, similar to that of the second stage, but larger; the *anterior sclerites* are rather irregular in form, with a moderately well-marked, backwardly directed dorsal apophysis, a broad triangular ventral apophysis, acute at its tip, and a rather stout, short, slightly curved anterior tooth, directed forward; the *intermediate sclerite* is moderately elongate, about one and one-third times as long as the anterior sclerite; the two lateral sclerites are connected ventrally

at about their middle by the rather narrow *sclerite of the salivary duct*; below the anterior sclerites are some small, rather irregular, accessory sclerites, including the usual *hypopharyngeal plate*; the *basal region* is large, the anterior border concave, and slightly oblique, the dorso-anterior angle of the basal region distinct, but rounded, the ventro-anterior angle acute, the *dorsal wing* broad at its base, but tapering rapidly to its tip which is acute, the *ventral wing* rounded at tip and approximately equal in length to the dorsal wing; in this stage the ventral surface of the pharynx is chitinised and pigmented.

The *anterior spiracles* (Fig. 55) (length 0·12 mm.) are rather broad, slightly explanate in some specimens, with nine or ten circular respiratory papillae, lying for the most part on the edge of the felt chamber, though one or two sometimes lie a little distance inward.

Puparium: length 5·5 mm., breadth 2 mm.; form, seen from above, cylindrical with rounded ends, the anterior extremity subcylindrical with prominent slender stigmata, the posterior extremity ovoid, terminating in a rather short, broad cylindrical projection, bearing at its end the *posterior stigmata* (Figs. 54, 58), separated by a rather shallow, acute-angled depression; ventral surface flattened, dorsal surface convex; the separation between the segments; colour, yellowish brown, surface, moderately polished; *pupal respiratory system* without prothoracic cornicles, comprising only the *internal spiracles* (diameter 0·192 mm.) which are rounded and bear 9–12 rows of respiratory papillae, of moderate size (Fig. 61).

Internal anatomy. The larva of *Frauenfeldia* is much less delicate and less transparent than that of *Plesina* and its allies.

The *hypodermis* is composed of cells which are, in general, much larger than in the stage II *Plesina* larva; the nuclei, which are frequently arranged in groups of two or three, are surrounded by what appear on surface view to be dense islands of cytoplasm, often with tail-like prolongations; seen in optical section, the hypodermal cells have the form of hanging drops or unduloids. The *imaginal discs* are much larger and more prominent than in *Plesina* and its allies; those of the anterior spiracles are large and spherical in form, lying just in front of the junction of segments I and II in the dorso-pleural region (diameter 60 μ). The *alimentary canal* (Fig. 66) is long, convoluted and sausage-shaped, without a distinct stomach in my specimens. The *oesophageal caecum* is unusually large, being equal in length to the ventral nerve cord; the *oesophageal valve* is piriform; no *gastric caeca* are present; the *posterior intestine* opens on the ventral side of segment XI; the *Malpighian tubes* are of the usual type, the recurrent section of the anterior pair being filled with carbonate granules; the *nervous system* is of the usual form; the cerebroid ganglia lie in segment III, while the ventral nerve cord extends posteriorly into segment V. The bulbs of the antennary and maxillary organs are unusually large; the *fat body* is composed of large polygonal mononuclear cells, with distinct outlines (diameter 25–35 μ), and comprises a pair of dorsal lobes in segment II and a pair of large, rather irregular lateral plates running through segments IV–IX inclusive. The

tracheal system is metapneustic; the tracheal trunks are robust (diameter 15–16 μ), the tracheoles simple, not moniliform; no distinct anterior commissure exists in this stage; the posterior commissure lies in the middle of segment XI; the *gonads* are very large (diameter 160 μ), embedded, as usual, in the fat body in segment VIII.

(2) *Cyrillia angustifrons* Rond.

Stage I. The only material representing this stage is a fragment of the skin attached to the buccopharyngeal armature. The structure of this fragment indicates that the larva is heavily armoured, resembling in its general characters that of *Frauenfeldia rubricosa*.

The *cuticle* of the anterior region bears numerous small oval, or triangular scales, brown in colour, and acute at the tip. Here and there are a few acute slender spines situated apparently on protuberances surrounded by a concentric group of overlapping scales (Fig. 53). These may possibly have a sensorial function.

The *antennae* (Fig. 50) (length 0·069 mm.) are quite different from those of *Plesina* and its allies, being very slender, elongate, and almost filiform. The organ arises from a rather broad circular area, surrounded by a low, dark brown chitinised ring.

The *buccopharyngeal armature* (Figs. 46, 47) (length 0·1394 mm.) resembles that of *Frauenfeldia rubricosa* in its general characteristics. It is composed of an *anterior sclerite* which is apparently bifurcate posteriorly, but appears to be simple anteriorly, though this cannot be determined with certainty until a complete specimen in this stage has been examined. Since the anterior sclerite appears to be homologous with that of *Plesina* and its allies, it may possibly consist of a pair of contiguous plates, which cannot be distinguished in a lateral view.

The *anterior sclerite* consists of an approximately quadrate base with rounded posterior angles, bearing small, backwardly directed, triangular projections in the middle of the dorsal edge, and an unchitinised, transparent, oval spot slightly above the middle of its lateral aspect. Anteriorly the sclerite is prolonged into two large, acute, curved teeth, the dorsal one being about three-fourths, and the ventral about one-half as long as the basal part of the anterior sclerite. Both teeth are acute at the tip, and separated by a rather broad, acute notch; just below the ventral tooth, and lying close to it, is a pale slender spine, about three-fourths as long as the lower edge of the ventral tooth. The *intermediate region* is elongate, dark brown, and rather heavily chitinised throughout, with the anterior extremity bending slightly ventrad, the dorso-anterior angle obtuse and rounded, the ventro-anterior angle, though rounded, acute. Posteriorly the width of the sclerite increases slightly to the level of the dorsal wing of the basal region, where it is about 1·4 times as wide as at the anterior extremity. The opening of the *salivary duct* is visible as a small rounded notch on the ventral side opposite the middle of the stem of the dorsal wing. The

basal region is well developed, the *dorsal wing* with a broad stem, of which the anterior border is slightly concave, the antero-dorsal angle approximately a right angle, the dorsal edge straight on its anterior two-thirds, but sloping gently backward and downward on its posterior third, the ventral edge almost straight; the *ventral wing* is also well developed, brown in colour, though with a pale ventral border, the postero-dorsal angle produced slightly upward toward the dorsal wing, the postero-ventral angle produced somewhat backward and downward to a point slightly beyond the level of the end of the dorsal wing, its tip rather broadly rounded.

Stage II: length 3 mm., width 0·5 mm. The larva in this stage is elongate and slender; the *cuticle* is thin, transparent, and practically naked, though a few small, slender, acute spines exist on the last segment; the *antennae* are small, inconspicuous and only feebly convex; the *tracheal system* is metapneustic; the *posterior spiracles*, which open on the posterior surface of the last segment, are very minute; the felt chambers, like the tracheal trunks, are very slender; each terminates in a pair of minute respiratory papillae, opening at the tip of a small conical protuberance; the *buccopharyngeal armature* (Fig. 48) is of the "*Plesina* type," but resembles that of *Frauenfeldia* and the undetermined species in its unusually elongate intermediate region, which is, however, completely fused with the basal region (total length 0·156 mm.); the *anterior* or *mandibular sclerites* are characterised by their short, broad, ventral apophysis and the exceptionally elongate, stout, anterior tooth, which is about the same length as the remainder of the mandible, almost straight and directed forward and downward; the *intermediate region* is also unusually elongate, about twice as long as its height at the anterior side of the notch into which the *salivary duct* opens; the *basal region* is well developed, the *ventral wing*, measured from the posterior side of the opening of the salivary duct, being almost two and a half times as long as the intermediate region; it is obliquely truncate behind; the ventral wall of the pharynx is pigmented, as in all the species of the *Plesina* group; the *dorsal wing* of the *basal region* is relatively short, about half as long as the *ventral wing* from the salivary opening to the tip.

Stage III. The description of the third-stage larval characters is based on a single puparium as no larvae have been obtained up to the present.

The buccopharyngeal armature (Fig. 56) (length 0·36 mm.) is of the type found in *Plesina*, *Styloneuria*, and *Phyto*, with a short intermediate region, distinctly separated from both the anterior and basal regions. The *anterior sclerites* are moderately large, with a short, curved, downwardly directed tooth and broad, blunt ventral apophysis; a large *hypopharyngeal plate* with two sensorial areas and a small *epipharyngeal plate* with two notches on the posterior side, probably containing sensorial organs, are also present; the *intermediate region* is T-shaped, the ventral area, which is the *sclerite of the salivary gland*, being fused with the lateral sclerites; the horizontal arm, or lateral sclerite, is slightly concave on its dorsal side; its posterior and anterior branches are of about the same length and the angles between their axes and the axis of the

ventral arm are about equal; but the anterior branch is rather broad and truncate at the tip, while the posterior branch narrows toward its extremity, which is acute; the *basal region* is well developed, the dorsal wing hardly narrowed at its origin, acute at its tip, and about the same length as the ventral wing.

The *tracheal system* is amphipneustic, the stigmata prominent, the tracheal trunks voluminous, much larger than those of the preceding stage.

The *anterior spiracles* (Fig. 49) resemble those of *Styloneuria*, having the respiratory papillae arranged along the periphery of the felt chamber; but in *Cyrillia* only five or six of these papillae are present. The *posterior spiracles* are situated at the end of the last segment; each presents three short, straight, respiratory slits (Fig. 52).

Puparium. The pupal respiratory system resembles that of the Sarcophagine flies in the absence of the prothoracic cornicle, only the internal spiracle being present. It is circular (diameter 0·084 mm.), with six radiating groups of respiratory papillae, comprising in all about forty elements; its diameter is about 95 μ.

Internal anatomy. The internal anatomy is very similar to that of the other species of the group; the *alimentary canal* is moderately convoluted but the *mid-intestine* is rather markedly dilated throughout the greater part of its length; the usual *oesophageal caecum*, opening into the left side of the oesophagus, is present; it is strongly dilated and almost globular in form in the only specimen available for microscopic examination; the *salivary glands* are of the usual form; the *fat body* is composed of plates of small mononuclear cells.

(3) *Species B.*

The only larva of this form so far discovered was found in a woodlouse (*Porcellio scaber*) received from the late Miss Marie Rühl and collected near Zurich, Switzerland, in 1920. The only material available for study consists of some fragments of the skin of the primary larva with the buccopharyngeal armature and the antenna, and the anterior spiracle of the third-stage larva.

Stage I. The *cuticular armature* of the primary larva is of a very unusual and characteristic type; the skin bears, not only numerous pigmented, acute scales, but groups of these arranged on protuberances (Figs. 69–72), which are almost globular in form and somewhat resemble the immature heads of a French artichoke; the spines on the outer sides of these curious organs are separate, elongate, and distributed rather irregularly; those on the inner side are short and broad, and arranged in overlapping rows. The *antenna* (length 0·0492 mm.) is slender and elongate, about fifteen times as long as its breadth at the base, inserted in a short, broad, collar-like base; the *buccopharyngeal armature* (Fig. 68) (length 0·18 mm.) resembles that of *Frauenfeldia*, but is a good deal stouter and more heavily pigmented; it is composed of an *anterior sclerite* and a *posterior region* comprising *intermediate* and *basal sclerites* fused together. The *anterior sclerites* (Fig. 62) consist of a suboval basal portion and two anterior

teeth, the dorsal being about three-quarters as long as the basal part of the sclerite, and about twice as long as the ventral tooth which diverges rather markedly from it; in the middle of the ventral edge of the *basal region* is a rather deep oblong notch; the dorsal edge presents, near the posterior extremity, a short, stout, sharp tooth, backwardly directed, below which, on the body of the sclerite, about one-third of the distance from the dorsal to the ventral margin, is a rather large, round, transparent spot. The *anterior sclerite* is probably composed of two closely appressed halves; but although the ventral tooth is clearly paired, it has not been possible to distinguish two dorsal teeth in the only specimen available. The *intermediate region* is elongate, somewhat more than twice the length of the anterior sclerite, about eight times as long as its height at the base, slightly curved about the middle, and having the anterior extremity rounded. The *basal region* is well developed, the *dorsal wing* about four-fifths as long as the intermediate region, and somewhat longer than the *ventral wing*. The dorso-anterior angle of the dorsal wing is distinctly produced, but rounded, the posterior extremity of this wing acute. The inner sides of the dorsal and ventral wings converge somewhat posteriorly.

The *anterior spiracle* of this stage III larva (Fig. 64) is enlarged distally, and bears 10–11 spherical respiratory papillae.

(*c*) *Species A group*

Species A (*undetermined*).

The identity of this species, which has been obtained up to the present only from woodlice collected in the cemetery of Haslar Hospital and at Caen, France, is as yet undetermined. It differs markedly both in structure and habit from all other species so far studied, and although it is presumably one of the Rhinophorine group, we have no clue to its identity. It will, however, be evident from the description and figures that, in the characters of the second-stage larva, it closely resembles *Frauenfeldia* and *Cyrillia*. Among the Rhinophorines which have been reared from woodlice *Stevenia* (*Ptilocerina*) seems most likely to be the adult of this form, but the perfect insect may still be unknown to science.

Stage I. The primary larva, as has been already pointed out, was erroneously attributed by the writer to *Phyto melanocephala* in a note published in 1917.

The best specimens available for description in this stage were obtained from woodlice dissected during the latter part of the winter; some growth had obviously taken place, so that the cuticular spines and scales, which probably lay close together in the newly hatched larva, were separated by more or less extensive areas of unprotected cuticle; in one or two specimens, which evidently died soon after entering the host, the scales overlap on the greater part of the body so as to form a close-set armour; this tends to form denser longitudinal bands opposite the sensorial setae described below (length: young larva, 0·6 mm.; well-grown larva, 1·2 mm.).

The *head* is of the usual type, quite small, without spines or scales, but bearing dorsally a pair of elongate, slender, whip-like *antennae* (length 0·0328 mm.). The thoracic and abdominal segments bear, over practically the whole surface, large numbers of rather small, broad, backwardly directed scale-like spines, light brown in colour, rounded anteriorly, acute posteriorly, those of the dorsal and pleural areas being, in general, somewhat larger than those of the ventral region. Toward the posterior borders of the segments, there occur, also, well-developed elongate slender spines, which may be sensorial in function and appear to be inserted at the top of conical eminences (Fig. 79), around which lie a set of overlapping scales. Segment I presents, on each side of the body, a pair of these spines situated fairly close together; segments II and III have two on each side of the pleural region; the abdominal segments present five on each side, though the spines they bear are much shorter, especially in the case of the ventral elements, and transverse the posterior extremity. The last segment (Fig. 75) is subglobular in form; it bears, on the ventral side of the posterior aspect, a pair of rather narrow cylindro-conical organs (Fig. 77) consisting of four segments, the basal one short and ring-like, the second somewhat larger, the third and fourth of about the same length each, equal to the combined length of the first two; on the dorso-posterior aspect are the posterior stigmata (Fig. 75), having the form of short, broad, conical projections, which like the ventral organs, are rather faintly pigmented, being yellowish brown in colour. Some short rod-like sensoria are also present, but their exact arrangement cannot be made out in the preserved specimens.

The *buccopharyngeal armature* (Figs. 74, 78) (length 0·144 mm.) is markedly different from those of all other known woodlice parasites, having two quite distinct *mandibular sclerites*, and having between the anterior end of the intermediate region and the mandibular sclerites a distinct bridge-like *accessory sclerite*, so that two articulations are present in the mouth-hook. It must, however, be noted that these articulations are not homologous with those present in the later stages of Tachinid larvae, which are respectively in front of and behind the true intermediate sclerite. Nevertheless, in its general conformation, the buccopharyngeal armature is undoubtedly similar to those of the other species of this group. It is heavily chitinised and dark brown in colour.

The *anterior* or *mandibular sclerites* are paired, distinctly separated at their bases, and diverging rather markedly at their tips. Each sclerite consists of a triangular basal portion which expands rather abruptly at its tip, where it bends downward and outward, dividing into two short, acute, markedly divergent teeth, of which the lower is slightly larger than the upper. Between the base of the mandibular sclerite and the anterior end of the intermediate sclerite is a *bridge-shaped sclerite*, whose lateral extremity is roughly triangular, the base ventrally directed, the apex joined to the corresponding piece on the opposite side by a narrow chitinous bridge. The lower edge of the buccal region is defined by a much narrower strip of chitin, which appears to begin just at the dorso-posterior angle of the mandibular sclerite, whence it runs ventrally

across the corner of the accessory intermediate sclerite just described, to the posterior border of the head, where it turns abruptly at right angles and runs transversely behind the buccal region to the opposite side, where it again turns upward. Finally, between the ventro-posterior angle of the mandibular sclerite and the transverse part of the ventral strip just mentioned, is an additional *accessory sclerite*, roughly triangular in form, lying parallel to the mandibular sclerite and slightly within it. The ventral angles of these sclerites are prolonged so that their tips almost touch in the mid-ventral line. It is possible that they represent the *hypopharyngeal plate* and that the dorsal bridge-like sclerite corresponds to the *epipharyngeal plate*, both of which are present in the later stages of most Tachinid larvae; but they apparently do not bear the usual sensorial organs present on these sclerites.

The *intermediate region* is straight, elongate and narrow, about twenty times as long as its dorso-ventral width, near the base; the opening of the *salivary duct* is not distinctly indicated; the *basal region* is rather feebly developed, the stem of the *dorsal wing* fairly heavily pigmented, but rather short and slender, the dorsal wing, which is curved, rather narrow, and feebly pigmented, with a somewhat ill-defined outline; the *ventral wing*, which is about as long as the dorsal wing, is also rather feebly chitinised.

Stage II. Only a single specimen of this stage has been obtained; it is not in sufficiently good condition to permit of a complete description. The *cuticle* is transparent, without any conspicuous spines or scales; the *tracheal system* is metapneustic; the *posterior spiracles* are of the usual type, inconspicuous, with slender felt chambers; the *antennae* are very difficult to see, but appear to be conical and elongate, seven or eight times as long as their width at the base (length 14 μ); the *buccopharyngeal armature* (Fig. 76) (length 0·168 mm.) is, in its general form, very similar to that of *Frauenfeldia*; in the only specimen available the *intermediate* and *basal* regions appear to be partially fused, but this may be due to a secondary formation of chitin along the pharyngeal wall in the later part of the second stage; the *anterior sclerites* or *mandibles* are roughly oblong in form, about twice as long as high; the dorso-anterior angle is prolonged into a straight, slender tooth, directed forward and slightly upward, a little constricted toward the base, with its lower edge straight, its dorsal edge rounded anteriorly, and its tip acute; the lower edge of the tooth is narrowly margined with dark brown, and this margin extends back a little distance along the dorsal edge; the *intermediate region* is like that of the stage II *Frauenfeldia* larva, but somewhat shorter and more robust, only a little over three times as long as its width at the broadest part; it tapers strongly toward the anterior end, which terminates opposite the posterior side of the mandibular sclerite; its posterior end is cut off obliquely as in *Frauenfeldia*, with the ventral angle obtuse and the dorsal angle acute and lying along the anterior edge of the basal sclerite; beneath the intermediate sclerites and fusing with their lower borders, is a broad plate, slightly more than half as long as the sclerites to which it is attached; this is the *sclerite of the salivary duct*, which

opens above its posterior border; a rather small *epipharyngeal plate*, bearing two small circular pale sensoria, lies above the anterior ends of the intermediate region, while a large *hypopharyngeal plate*, bearing a pair of large oval sensorial areas, lies on the ventral side of the same plate; the *basal region* is irregular in outline and rather feebly pigmented; it is roughly triangular in form; the anterior border is oblique, the ventro-anterior angle irregularly acute, the dorso-posterior angle obtuse; the dorsal and ventral wings are short and irregular in form; the ventral pharyngeal wall of the basal region is unpigmented, only the lateral plates being distinctly visible.

Internal anatomy. The immature larvae available are unsuitable for detailed anatomical studies. However, in its general characters, this species seems to agree with the other members of the group.

The *digestive system* (Fig. 73) is of the normal type; just behind the pharynx arises the *oesophageal caecum* containing one or two of the granules described in *Plesina maculata*, and which, it may be added, have a marked affinity for chromatin stains; the *oesophageal valve* is globular and of the normal structure, but broadly joined to the mid-intestine; no *gastric caeca* are present; the *mid-intestine* is moderately elongate, and consequently only slightly convoluted; the anterior two-thirds is irregularly cylindrical, with a rather thick epithelial wall composed of numerous cells, with rather small nuclei, some undergoing mitotic division; the posterior third is abruptly enlarged so as to form a sack-like *stomach*; at the anterior end of this the epithelium seems rather markedly thickened, so as to form a *rudimentary valve*; in the sack-like part of the mid-gut the cells are flattened, forming a pavement epithelium; generally speaking, the nuclei in this region are arranged in pairs, the two members of which very often touch; it is therefore possible that the cells in this region are binucleate though their boundaries are indistinct in my material. Posteriorly the stomach narrows abruptly and passes into a short cylindrical section terminating in a narrow neck, which is apparently closed. Behind this is a slight globular enlargement, into which open the *Malpighian tubes*; and from this point continues the narrow *hind-intestine*, which opens in the last segment. The *salivary glands* are of the usual type, simple and tubular in form, rather markedly enlarged anteriorly, and composed of rather large convex mononucleate cells with vacuolated cytoplasm and prominent nuclei; each opens into a short slender duct, which fuses with its fellow to form a median canal opening into the pharynx opposite the stem of the dorsal wing of the basal sclerite. The *Malpighian tubes* are also of the usual type, and comprise an anterior pair, whose recurrent portion is enlarged, with thin walls, and is normally filled with concretions of calcium carbonate, and a posterior pair, much shorter and uniform in structure; the cells of the Malpighian tubes are apparently mononucleate. The *fat body* is composed of plates of small cells, which are apparently mononucleate. Various phases of mitotic division were observed here and there in this tissue. The *nervous system* is of the usual type with a pair of globular cerebroid ganglia and an unsegmented ventral mass.

(*d*) *Rhinophora lepida* Meig.

An adult of this species (Figs. I_1, III_3) was found with two adults of *Frauenfeldia rubricosa*, at Farnham Royal on July 1st, 1929, in a closed cage in which specimens of *Porcellio scaber*, collected in north-western France, had been confined with pieces of bark. There is a strong presumption that it emerged from a dead woodlouse, but definite proof of this was not secured, and since then the species has not been reared. Species A or B may perhaps be *Rhinophora lepida*.

(*e*) *Summary*

As we have already noted, the dipterous parasites of woodlice fall naturally into three main groups, based mainly on the structure of the buccopharyngeal armature of the first-stage larva. In the forms constituting the first group (*Plesina*, *Melanophora*, *Phyto*, *Styloneuria*) the buccopharyngeal armature is slender and rather delicate in its general construction, having only a single articulation and with an anterior region rather short, subquadrangular in form, bearing several more or less distinct, small teeth; in those of the second group (*Frauenfeldia*, *Cyrillia*, species B) the buccopharyngeal armature is stout, heavily sclerotised and deeply pigmented, having only a single articulation, but with one large and one rather small tooth; finally, in the unique representative of the third group (species A), the buccopharyngeal armature is elongate, but stoutly constructed, has two articulations, and two quite distinct anterior sclerites, each ending anteriorly in two teeth. In most of the species of the first two groups, the anterior sclerite is composed of distinct halves, fused at the base; these are probably the homologues of the two distinct sclerites in species A. If this view is correct, the anterior sclerites in all of these forms may be the homologues of the paired mandibles in the later stages, and these parasites would then be morphological allies of the genus *Sarcophaga*, which is one of the few Muscoids possessing distinct paired mandibles in the first larval stage. The dorsal oesophageal caecum, present in the larvae of these parasites, also exist in the Sarcophagine, as well as in the Miltogrammine flies and a number of the non-parasitic Muscids.

The second-stage larvae of the species studied do not arrange themselves in quite the same way as the first-stage larvae. *Plesina*, *Melanophora*, *Phyto*, and *Styloneuria* fall naturally together, owing to the similarity of the buccopharyngeal armature; but the second-stage larvae of *Cyrillia angustifrons* and species A must also be grouped with them. *Frauenfeldia rubricosa* and species B must be placed together because of the existence of two articulations in the buccopharyngeal armature, which are very similar in their general form.

The third-stage larvae form a homogeneous group. *Melanophora roralis* is distinguished by the fact that it has only a single articulation in the buccopharyngeal armature; *Frauenfeldia rubricosa*, by the unusually elongate intermediate region; but the other species known in this stage differ very little and are exceedingly difficult to distinguish.

The general effect of the successive stages is thus that of individuals of independent origin, constituting not only a number of distinct species, but several fairly clear-cut, morphological groups, which gradually become more and more similar in structure as they develop, under the influence of the common and rather unusual environment to which the larvae are confined. This morphological "convergence" in the later stages is, however, a fairly common phenomenon among the parasitic Diptera.

V. The biology of the parasites of woodlice

(1) *Life history*

Little is known in regard to the habits of the parasites in the adult stage. *Plesina maculata* has been taken in Britain in July, August, and September, *Rhinophora lepida* and *Styloneuria discrepans* in July and August, *Melanophora roralis* in June, *Phyto melanocephala* in September, *Ptilocerina atramentaria* from the end of May on into July. I have taken *Stevenia umbratica* commonly on maize in the early summer in the Mediterranean region. So far as the records go they seem to indicate that the adults occur in the field during a considerable period. Judging from laboratory experiments they are not, however, exceptionally long-lived species. Nothing has been found to suggest that any of the species has more than a single generation per annum. On the other hand, it is clear that the length of time required for the larval development, and, consequently, the moment of the emergence of the adult, depends to a very considerable extent on the conditions under which the host is living, and it seems probable that the reason for the occurrence of the adult parasites in the field over so long a period is due mainly to their very irregular emergence.

I have not observed the mating of any of these species in the field, but *Plesina maculata*, *Styloneuria discrepans*, and *Melanophora roralis* have been found to copulate readily in the laboratory when confined in small glass cages. A pair of *Melanophora roralis* remained in copula for at least an hour and a half; a male of *Plesina maculata* remained attached to the female for about three days, eventually dying in this position, but this was apparently due to some abnormality in the genitalia, and was not observed with other individuals of this species.

Because of the rarity of the parasites and the irregularity in their development, the attempts to obtain mated females were, in general, unsuccessful. A mated female of *Styloneuria discrepans* died without ovipositing. A specimen of *Melanophora roralis* observed in copula on February 9th, 1931, but which by February 13th, 1931, had failed to oviposit, was placed on the latter date with some woodlice in the hope that their presence would stimulate reproduction; but no eggs were obtained, and on February 19th, 1931, the female, which had become very feeble, was found to have been half-devoured by the woodlice during the night. However, early in 1931, a female of *Plesina maculata*, left in a cage with a male without special attention, was found about two weeks later to

have deposited a considerable number of elongate, fusiform, thin-shelled eggs on the paper bottom of the glass cage, in which she was confined, and about a week later it was found that these eggs had hatched, producing pigmented and armoured larvae, which died and dried up after emerging, as no woodlice were present in the cage.

The existence of a group of Tachinids having a reproductive habit of this kind had not hitherto been suggested, and no such category is provided by J. Pantel in his classification of the types of reproduction. The writer of this paper had noticed, in dissecting adult females from collections and from the field, that a number of species were never found to contain anything but undeveloped eggs and had therefore suspected that these were deposited in an immature condition, though they appeared to differ little from the eggs of species producing unarmoured larvae ready to hatch; but he had never succeeded in inducing any of these forms to oviposit, though several, as for example *Stevenia umbratica*, were collected alive and kept in confinement for considerable periods.

The following species are believed to belong to this group:

Anthracomyia melanoptera	*Plesina maculata*
Frauenfeldia rubricosa	*Ptilocerina atramentaria*
Melanophora roralis	*Rhinophora lepida*
Morinia nana	*Stevenia umbratica*
Phyto melanocephala	*Styloneuria discrepans*

Other species belonging to these and allied genera, included in Villeneuve's Rhinophora section of the Rhinophorinae, probably have similar habits.

The complete diagnosis of the group cannot be given until sexually mature adults of all the species are available for study. The main characteristics of the reproductive systems of the male and female of *Frauenfeldia rubricosa* Meig., are, however, shown in Figs. 12 and 14. The ovaries of the female (Fig. 12) are of moderate size, with a moderate number of ovarioles and ovarian follicles. Three darkly pigmented, small, spherical spermathecae are present. The accessory glands are moderately elongate, being about twice as long as the spermathecae with their ducts. The uterus is not very profusely provided with tracheae and is not elongate in the mature female, containing only a relatively small number of eggs, elongate and spindle-shaped in form, with a rather thin, lightly sculptured, transparent, colourless cuticle; these eggs are laid in a very early stage of development and do not hatch for 10–14 days after deposition; no piercing ovipositor is present.

In the male (Fig. 14) the testes are rather elongate, red in colour; two accessory glands are present; they are well developed and cylindrical in form.

No individual belonging to any of the species discussed in this paper has as yet been observed during oviposition, either in the laboratory or in the field. It is, however, evident from the experiment with *Plesina maculata*, that the presence of the host is not necessary in order to induce oviposition, since, in the case mentioned, the eggs were disseminated haphazard on the filter paper

forming the floor of the cage. It does not seem likely that the egg is normally deposited upon the body of the woodlouse. The individuals of the species most frequently infested by these parasites (*Porcellio* spp. and *Oniscus asellus* L.) pass the greater part of their lives in protected situations, under stones or bark, and are thus inaccessible to direct attack by a parasite without a piercing ovipositor. Eggs destined to be attached to the host usually exhibit some correlated peculiarity in form or structure, such as a plano-convex form with an adhesive under surface (*Tachina larvarum* L., etc.), or an adhesive pedicel (*Carcelia cheloniae*, etc.). There is nothing of the kind in the eggs of the parasites of woodlice. Eggs without means of fixation deposited directly on the body of the host normally contain larvae ready to hatch, whereas the eggs of the Rhinophorines are deposited in a very immature condition and, judging from the case of *Plesina*, develop very slowly. Finally, the cuticular armature exhibited by several of the primary larvae of the group (*Plesina maculata*, *Cyrillia angustifrons*, *Frauenfeldia rubricosa*, species A and B) is, so far as we know, found only in forms which either lie in wait for their hosts (Echinomyiine Tachinids) or penetrate into their habitats in search of them (Dexiids). The most reasonable hypothesis as to the reproductive habit of the dipterous parasites of woodlice is, therefore, that they deposit undeveloped eggs in the crevices of the bark or at the edges of stones, under which colonies of the hosts are assembled, and that the larvae hatching from these eggs go in search of them.

The entrance of the larva into the woodlouse has not been observed. Since the host is heavily armoured, penetration is hardly possible except through the relatively soft cuticle separating the ventral sclerites or around the bases of the appendages. The majority of the larvae are attached to the cuticle in the posterior part of the body, generally along the pleural line or to the integument separating the gills. Generally speaking, they appear to lie in delicate integumental sheaths, resulting from the ingrowth of the hypodermis around the posterior extremity, which is left lying in the opening through which the primary larva made its entrance.

The exact position of the larva is, however, not always easy to determine with certainty, partly because the woodlouse is somewhat inconvenient to dissect and partly because it contains a considerable amount of connective tissue. On several occasions larvae have been found which appeared to have no connection with the exterior. In the hope of getting an accurate idea of the respiratory relations of the parasite, batches of *Porcellio* were placed in various substances which penetrate into the tracheae, such as solutions of Sudan III and other dyes, clove oil, benzene, chloroform, and so forth. Other lots were killed in hydrogen sulphide, after which they were dissected, the parasite larvae removed and washed, and opened in lead acetate solution. When this gas has penetrated into the tracheae a precipitate of lead sulphide forms in the tissues. In the case of the parasites of woodlice, no positive results were obtained by any of these methods. That the larvae often do lie in the

integumental funnel with their posterior spiracles in direct communication with the external air, there is no doubt (Fig. 11). On the other hand, the felt chambers of the posterior spiracles are extremely slender and somewhat inadequate in appearance, while the skins of the secondary larvae of a number of species (*Plesina* and allies) are very thin and delicate. It is possible that the larva obtains the greater part of its oxygen supply from cutaneous respiration. A number of second-stage larvae of *Plesina maculata* were therefore studied by Dr W. H. Thorpe, who tested them by the "biological indicator" method, using a culture of *Polytoma* (Thorpe, 1932), and showed conclusively that cutaneous respiration was going on.

Like other Tachinids, these parasites pass through three well-differentiated larval stages[1]. No accurate information regarding the relative length of these stages has yet been obtained. It is, however, evident that in all the species so far studied, with the exception of the species A, by far the greater part of the larval life is spent in the second stage. Species A has always been found in very small specimens of *Porcellio*, in which it hibernates as a primary larva; but all of the others studied pass the winter in the second stage, in which they may be found during the greater part of the year. It seems, therefore, that the first stage is of short duration, and that the larva moults within a week or so after it has penetrated into its host. During the first two stages the larvae appear to feed on the blood of the host, and during this time their increase in size is very slow. As soon as it reaches the third stage, however, the parasite larva begins to attack the tissues and organs of the woodlouse, and increases rapidly in size. When it has finished feeding, practically nothing but the cuticle is left.

The full-fed parasite larva invariably pupates within the empty skin of the host, usually with its anterior end directed forward, the head and some of the anterior segments of the woodlouse becoming detached when the fly emerges (Fig. 32).

The habit of hibernating in the second instar in the body of the host is not uncommon in Tachinid larvae. In many such cases the host enters the hibernating phases in spite of the fact that the weather is warm and the insect is itself far advanced in its development, and has indeed definitely ceased to feed. Such, for example, is the case with *Pyrausta nubilalis*, which goes into hibernation in southern France toward the end of August, and cannot usually be induced to complete its development, even though it is kept continuously at a high temperature thereafter.

It is an interesting fact that the parasites of these hibernating insects are affected by the condition of their hosts. The Tachinids, *Zenillia roseanae* and *Masicera senilis*, remain quiescent in the body of *Pyrausta* as second-stage larvae until the host resumes its development the following spring. Similarly, the Ichneumonid, *Angitia punctoria*, remains in the first larval instar until the

[1] A thin, delicate membrane lining the puparium is the result of an ecdysis occurring not long after pupation; but the stage preceding this is to be regarded rather as a pupal than a larval instar.

Pyrausta caterpillar has emerged from its diapause. This is obviously not due to a lack of food, for the larvae of *Eulimneria crassifemur*, living in exactly similar caterpillars in the same environment, complete their larval development, destroy the host, and spin their cocoons in the autumn. It does not seem to be due to the invariable or inflexible character of the larval development in *Angitia*, *Zenillia*, and *Masicera*, for *Zenillia* and *Angitia* were reared in the laboratory through several generations in succession by Dr H. L. Parker and the writer. The arrested development of the parasite larva must therefore be determined either by the external conditions acting through the host larva as a physical medium, or by the physico-chemical condition of the body fluids of the host larva itself.

Various authors have suggested from time to time that between an endoparasitic larva and the organism of its host there is a physiological relation, somewhat similar to that which exists between an organ of the host and the rest of the body. The present writer is inclined to think that the analogy between these cases is rather superficial. It does, however, seem natural to suppose that between such potentially different and ontogenetically isolated systems, as the imaginal histoblasts (including the genital rudiments) of the caterpillar and the larval tissues, there might be somewhat the same relation as there is between the parasite larva and the organism of the host; and if there is any ground for this comparison we might be tempted to find in it at least the beginning of an explanation of the parallel arrest of development in the case we are considering. If the cause of the arrest of development in host and parasite is the same, it seems that this cause is to be sought in the condition of the blood of the host organism as a nutritive medium. Some of the parasites that hibernate in the living host depend on the blood not only for food, but also for oxygen, as, for example, *Fortisia foeda* in *Lithobius forficatus*, *Rhacodineura antiqua* in *Forficula auricularia*, *Angitia*, *Eulimneria*, and *Apanteles* in *Pyrausta nubilalis*, but there are others, such as *Erynnia nitida* in *Galerucella luteola* and *Masicera senilis* in *Pyrausta nubilalis*, whose larvae have direct access to atmospheric air during the diapause. In such cases the parasite is in physiological relation only with the blood of the host, or with the semi-fluid contents it imbibes from tissues, such as the fat body, in which it is embedded. If during the condition of diapause the blood becomes unsuitable as a nutritive medium, so that the development of the imaginal histoblasts of the host is inhibited, it is quite understandable that the development of the parasite larva should also be inhibited. On the other hand, when after the long response to cold or desiccation that commonly precedes reactivation, the development of the host is resumed, it is natural that the development of the parasite is also accelerated.

It is, of course, possible that we have in this case simply a parallel arrest of development due to the action of some environmental factor acting simultaneously on the host insect and the parasite within it; but that both are really in a diapause is certain, for exposure to high temperatures has often no

more effect on the parasite than it has on the host. It seems, therefore, that a careful physico-chemical study of the blood of insects in diapause as compared with those whose development is proceeding normally, might throw some light upon the difficult problem of hibernation.

As I have already said, the character of the diapause varies considerably in different parasites living in the same host. Among the parasites of *Pyrausta nubilalis*, *Macrocentrus* hibernates as an egg, *Angitia* and *Apanteles* as primary larvae, *Zenillia* and *Masicera* as second-stage larvae, *Eulimneria* as a pupa within the cocoon. The larva of species A, parasitic on woodlice, passes the winter as a primary larva, whereas all of the others hibernate in the second stage. A similar variation occurs in free-living species. It is therefore obvious that the condition of diapause is a good deal more complex than the simple cessation of development induced by low temperature, and that the physiological state of the organism concerned is of very great importance. In some species, such as the Corn Borer and Codling Moth, premature exposure to high temperatures usually produces death. In others, such as *Eulimneria crassifemur*, it causes an acceleration of development, which nevertheless remains definitely slower than the development of the non-hibernating generation. The diapause of the woodlice and their parasites is of the latter type. Under normal conditions, development proceeds slowly in the summer and autumn, is interrupted by the winter and completed only in the late spring and early summer, but when specimens are brought indoors early in December, kept in a warm room and provided with plenty of food and moisture, their development is definitely accelerated so that female woodlice bearing eggs, full-grown parasite larvae, and adult flies are obtained as early as the middle of January. Adults of *Styloneuria discrepans*, *Melanophora roralis*, *Frauenfeldia rubricosa*, and *Plesina maculata* have all been obtained in this way several months before they normally appear in the field. Up to the present, however, I have found nothing to indicate that these parasites ordinarily pass through more than one generation per annum, though they may at times require more than a single year to complete their development.

The following table, summarising the results of dissections from all sources, gives some indication of the relative frequency of the various species in the whole area from which collections were made:

		%
Plesina maculata	62	38·0
Styloneuria discrepans	39	24·0
Frauenfeldia rubricosa	23	14·0
Species A	15	9·2
Phyto melanocephala	13	8·0
Melanophora roralis	7	4·3
Cyrillia angustifrons	4	2·4
	163	

(2) *Effect of the parasite on the host*

So far as I have noted, the presence of the parasite has no marked general effect on the structure of the host. Parasitised specimens do not appear to

differ in coloration, sculpture, or general external conformation from normal individuals. In some districts, various colour forms of *Porcellio scaber* exist, some specimens being red, or variegated with yellow; but the pigmentation does not seem to be affected in any way by the presence of the parasite. Perhaps the parasitised woodlice tend, on the whole, to be somewhat smaller than the unparasitised specimens, but the variation among normal individuals at any given moment of the year is so considerable that it is difficult to be certain of this point.

A definite effect is, however, produced by the presence of the parasite in the reproductive system and secondary sexual characters of the host.

In the various species of woodlice examined during the work, secondary sexual characters are not very well marked. The last pair of legs in some species of *Porcellio*, such as *P. pictus* Brandt, is more heavily built than in the female, having the carpal joint considerably dilated, but in *P. scaber* and *Oniscus asellus* they differ little, if at all. In *Porcellio scaber* the outer ramus of the uropod is comparatively larger in the male than in the female, and in all species the first pair of pleopods of course differ greatly in the two sexes, being merely rounded in the female, while they are largely developed in the male to form copulatory organs. Before and after the period of oviposition and incubation there is therefore little difference in external morphology between the two sexes. At the ecdysis preceding the deposition of the eggs the normal fertilised female undergoes, however, a marked morphological change, owing to the development of the ventral plates or oostegites, forming the brood pouch, or marsupium. These form beneath the hypodermis of segments II-IV inclusive, and remain folded upon themselves until the moult before the eggs are deposited, when they expand and overlap to form a delicate, thin-walled, but efficient brood pouch. In this pouch the eggs are deposited and develop in an albuminous liquid. After the moult referred to the ventral surface of the segments comprising the marsupium becomes extremely thin and delicate, and in the middle of the ventral surface of each of the four segments comprising it, there appears a peculiar, elongate, conical projection known as the cotyledon. These organs, according to Vandel (1925), are filled with lacunae and richly vascularised, and, when fully developed, folded on themselves in the marsupium cavity. Vandel states that the cotyledons increase in length as the embryos develop. He maintains that they have a nutritive function and secrete food material for the embryos. Alceste Arcangeli (1929), however, rejects this idea on the ground that the eggs have an ample provision of vitellus. He agrees that the cotyledons, together with the walls of the marsupium, secrete the liquid in which the eggs are immersed, but considers that this is chiefly of value in providing a liquid medium for the development of the ova. Vandel suggests further that the cotyledons provide an extra supply of oxygen for the female and the incubating eggs; but Arcangeli, while admitting the possibility of this function of the cotyledons, thinks that the extra supply of oxygen is of value only to the eggs and is not required by the incubating female.

In spite of the abundance of woodlice, their life history and reproductive habits have not been intensively studied. A very remarkable mode of reproduction was attributed to these animals by Schöbl, who asserted (1880) that the eggs, after passing from the ovary into the oviduct, issue from the latter by a special opening through which they fall into the body cavity, whence they eventually pass into the marsupium through a broad orifice in the ventral surface of the body, between the fifth and sixth thoracic segments. Leichmann (1891) and Calman in the Oxford *Treatise on Zoology* (1909) refused to accept the account given by Schöbl. It has, however, according to Vandel (*loc. cit.*) been repeated by a number of authors, including H. Friederich (1883), Rosenstadt (1888) and Verhoeff, the latter author in a paper written as late as 1920. It is now known that the statements of Ströbl were incorrect.

The ovaries (Fig. IV_2) are a pair of flattened, elongate, ribbon-like organs, rounded at the ends, and attached to the body wall by suspensory filaments. The wall of the ovary is thin, formed of a multitude of small cells, without distinct boundaries, but with very distinct, numerous nuclei. This is, according to Vandel, a germinal layer. From it there arises the ovules, which appear to develop in *Porcellio scaber* mainly along the edge of the ovary from which the oviduct arises, though, in his description of *Metaponorthus pruinosus*, Vandel seems to suggest that the ovules arise mainly on the outer side. Each ovule is surrounded by a layer of follicular cells which later disappear, and has its cytoplasm filled with granules of vitellus, which become more abundant in normal specimens as the ovules increase in size. The oviduct opens on the ventral side of the fifth segment, not far from the attachment of the legs. It is lined with a single layer of epithelial cells, underlying a layer of connective tissue. Accessory glands open into the oviduct in some species (*e.g. Trichioniscus* spp.). The inner wall of the oviduct secretes a chitinous lining, which is continuous with the cuticle of the body wall, and which is closed at its inner end. In virgin females the chitinous lining is thin and forms a kind of *receptaculum seminis*.

Copulation appears to take place at night and has been observed only by Schöbl (*loc. cit.*), who states that this is a very rapid process. The spermatozoa are injected into the seminal receptacles, of which the inner ends rupture under the effect of the secretion of the accessory glands, permitting the spermatozoa to reach the upper part of the oviduct where they assemble in a whitish, ball-shaped mass. Later they spread through the ovary in which the fertilisation of the ovules takes place. A moult then occurs, after which the sack-like lining of the oviduct is replaced by a solid chitinous rod blocking the cavity and preventing the introduction of spermatozoa.

According to Verhoeff, reproduction never begins in the year in which the females leave the marsupium. The reproductive period begins in April and ends in September, and may include as many as three broods, preceded, separated or followed by moults, as follows: 1st moult, 1st brood; 2nd moult, 2nd brood; 3rd moult, 3rd brood; 4th moult, 5th moult. Large Isopods,

according to Verhoeff, may live three years and produce seven or eight broods.

Oviposition, according to Nemec (cited by Vandel, *loc. cit.*) coincides with a moult. The eggs descend through the oviduct or fall directly into the marsupium, the whole process being very rapid and occupying (Leichmann, cited by Vandel) at most, about two minutes. The incubation period in *Porcellio scaber* lasts from 49 to 102 days. After the larval woodlice have issued from the marsupium, the female again moults, losing the cotyledons and oostegites and resuming its previous characteristics.

The male reproductive organs (Fig. IV_1) are paired. On each side of the body are three elongate, fusiform utricles, containing developing spermatozoa and homologous with testicles. Suspensory ligaments attach these to the body wall; these ligaments often contain masses of cells, considered by Lereboullet (Vandel, *loc. cit.* p. 320) to be testicles and figured as such by Webb and Sillem (1906, Fig. 21) in their monograph of *British Woodlice*. The three genuine testicles open separately in the outer side of an elongate, cylindrical *seminal vesicle*, of which the wall is formed by large glandular cells. During the period of sexual activity this is filled with packets of spermatozoa. The seminal vesicle opens into a *vas deferens*, from which it is separated by a muscular sphincter. The *vasa deferentia* unite to form a common duct in the seventh abdominal segment.

As is well known, in many organisms there is a very clear relation between the nature and development of the gonads and the secondary sexual characters. Many observers, among whom Alfred Giard was one of the earliest, have also noted that in Arthropods of various types, individuals infested by internal parasites may exhibit at the same time degenerative changes in the structure of the reproductive organs and modifications in the secondary sexual characters. It is natural to suppose that the alterations in the secondary sexual characters are in some way dependent on the degenerative changes in the gonads, which appear to be determined by the parasites. In Vertebrates this actually appears to be the case. In Arthropods it was long assumed to be so. The experiments and observations carried out in recent years have not, however, provided much support for this view. They tend to suggest, that in insects at least, the development of the secondary sexual characters is independent of the sexual glands and that the action of the parasite produces a more general disturbance of metabolism, which affects both the structure of the gonad and the secondary sexual morphology.

It is of particular interest to note, in this connection, that in both Crustacea and insects, parasitised male individuals tend to assume female characteristics. In spider-crabs (*Inachus*) infested by *Sacculina neglecta*, for example, the male may actually develop the four pairs of ovigerous appendages characteristic of the female. It was suggested that this was due to the fact that the roots of the parasite absorbed from the blood the substances withdrawn from it by the ovary of a normal female, and thus stimulated the production in the

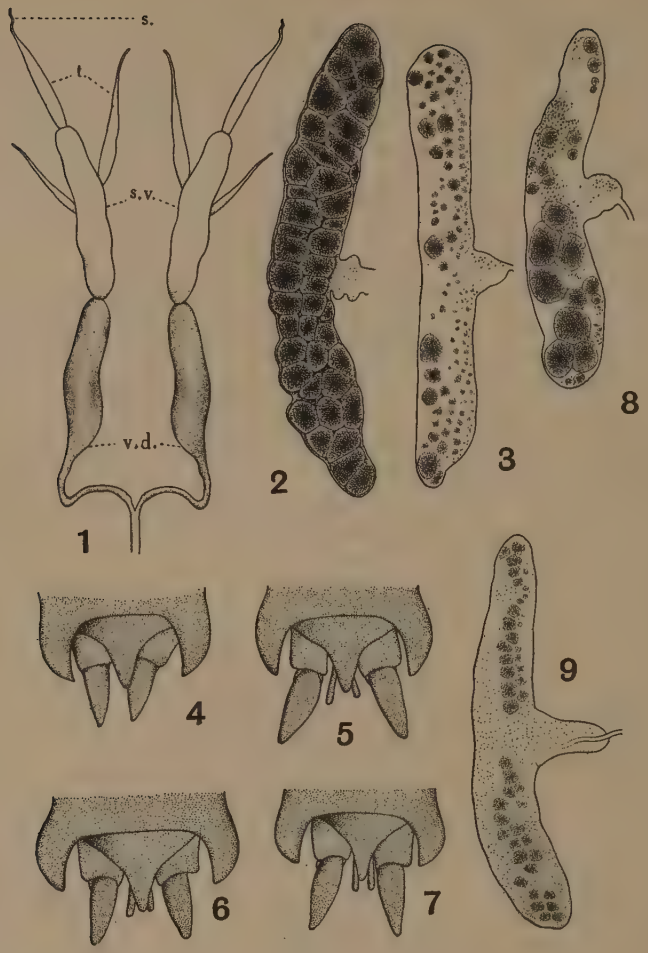


Fig. IV.

1. *Porcellio scaber* L. Reproductive system, male: *s.* suspensory filaments; *t.* testes; *s.v.* seminal vesicles; *v.d.* vasa deferentia.
2. *Porcellio scaber* L. Ovary, unparasitised specimen, dissected March 7th, 1932, showing form and disposition of ovules in normal individual.
3. *Porcellio scaber* L. Ovary, specimen parasitised by larva of *P. maculata*, Stage II, showing degeneration of ovules, relative absence of vitellus and irregular arrangement.
4. *Porcellio scaber* L. Posterior extremity, unparasitised female.
5. *Porcellio scaber* L. Posterior extremity, unparasitised male.
6. *Porcellio scaber* L. Posterior extremity, female parasitised by *P. maculata*, Stage II.
7. *Porcellio scaber* L. Posterior extremity, male parasitised by *P. maculata*, Stage II.
8. *Porcellio scaber* L. Ovary of another specimen parasitised by *P. maculata*, showing partial degeneration of ovules and irregular form.
9. *Porcellio scaber* L. Ovary regenerating after oviposition to show regularity in form and arrangement of developing ovules as compared with parasitised specimens.

body of substances concerned in the development of the secondary sexual characters.

The foregoing remarks constitute only the barest summary of some of the main facts that have been discovered in regard to this subject, around which a vast literature has now accumulated.

In a note dealing with the parasites of woodlice, published in 1917, the writer pointed out that these parasites exert a definite effect on the reproductive system and that this extends to both the gonads and the secondary sexual organs.

No direct observations on the entrance of the parasite larvae into their hosts have yet been made. The infested woodlice found in the autumn, though very variable in size, are, however, usually quite well developed; and up to the present, really small individuals have been found to be unparasitised except in areas inhabited by the undetermined "species A," of which the larvae have almost always been found in very small specimens of the host. Woodlice grow rather slowly. The available data therefore suggest that individuals are often fairly well developed at the time the parasite enters.

Furthermore, although the effect produced by the presence of the parasite on the metabolism of the host may fairly rapidly manifest itself in the internal organs, no change in the external or skeletal structures can take place until the time of the moult; and as we have already seen, moulting does not occur very frequently and may perhaps be somewhat delayed by the feeding of the parasite larva. The parasite larva develops very slowly during the winter and does not enter on a phase of rapid growth until it has reached the third stage, which occupies only a relatively short period.

Extensive degenerative changes in the organs of the host, and particularly in the external organs, are therefore hardly to be expected. At all events up to the present, no modifications in the structure of the infested male woodlice have been observed. The accompanying figures (Figs. IV_4–IV_7, V_1–V_{12}) of the uropods and telson of parasitised and unparasitised individuals of *Porcellio scaber* show no definite differences between the two categories. The internal reproductive organs of parasitised males appear to be normal in form and structure, and contain an abundant supply of spermatozoa. Nothing suggesting an assumption of female morphological characters by infested males has been observed.

During the winter season, infested females are externally indistinguishable from normal females. The ovaries are, however, almost always more or less abnormal in structure. The normal ovary of the over-wintering female is usually rather thick, divided into distinct, clear-cut areas, representing the ovules, which are whitish or yellow and opaque, owing to the presence in the egg cells of large masses of fat globules. In parasitised specimens the ovaries (Fig. IV_3, IV_8) are usually thin and flattened and more or less transparent owing to the absence of fat globules. As the parasite larva develops, the ovaries become more and more transparent until eventually they become practically

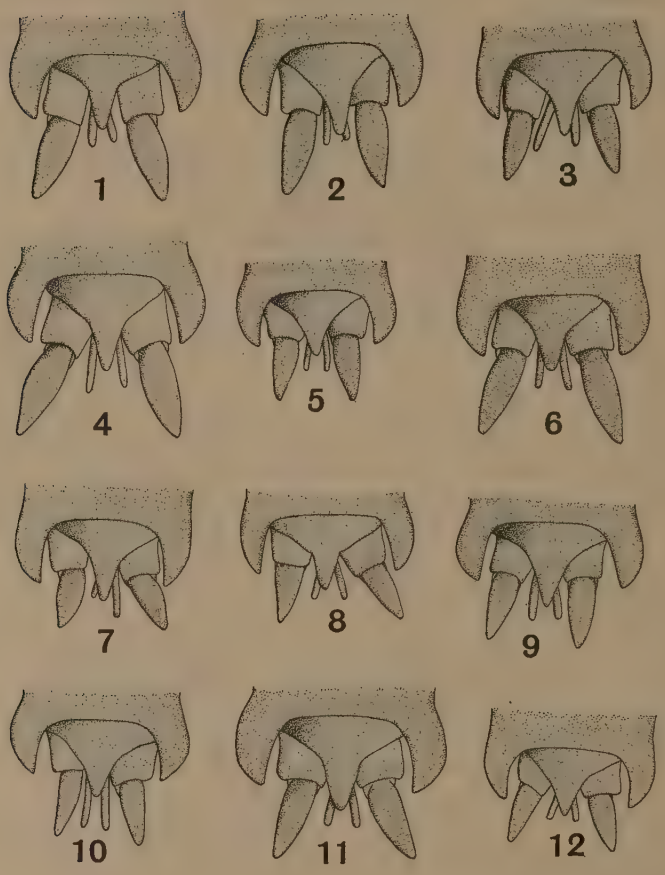


Fig. V.

1, 2, 3. *Porcellio scaber* L. Posterior extremity, unparasitised males.
4, 5, 6. *Porcellio scaber* L. Posterior extremity, males parasitised by *P. maculata*, Stage II.
7, 8, 9. *Porcellio scaber* L. Posterior extremity, unparasitised females.
10, 11, 12. *Porcellio scaber* L. Posterior extremity, females parasitised by *P. maculata*, Stage II.

invisible when *in situ*. At this stage there is a general superficial resemblance between the ovaries of the parasitised females and those of very young unparasitised females (Fig. IV_9). In slightly older normal specimens, however, the ovules, though transparent, usually form a complete cellular pavement, filling the whole ovary from side to side and from end to end, precisely as in full-grown normal individuals. In the well-grown parasitised specimens, however, the ovary appears often to be partly empty, containing only scattered ovules, usually circular instead of polygonal in form, and with transparent cytoplasm, scattered about through its cavity. The form of the ovary itself often becomes somewhat abnormal (Fig. IV_8). The macroscopic and histological appearance varies considerably, however, from specimen to specimen. The general impression derived from the examination of parasitised specimens in various stages of development is that the ovaries of parasitised specimens are either well developed at the moment of infestation, or continue to develop in a fairly normal manner for some time afterward. The larva of the parasite appears, however, to require a large amount of fat for its development and seems to derive this from the blood of the host; but as it develops it apparently begins to draw on the supply of fat contained in the ovaries themselves. As a result of this process the individual ovules tend to shrink and become transparent; the ovary itself, emptied of part of its contents, tends also to contract, and to become more or less irregular in form. Toward the end of the winter, parasitised females can almost always be recognised by the examination of the ovary, even though the parasite larva has not yet been found.

The effect produced by the presence of the parasite on the female gonads is thus profound. It does not, however, seem to be irreparable. On several occasions specimens of *Porcellio scaber* have been found in which the parasite larva has died in the second stage, after attaining a considerable size, and in some of these individuals the ovaries seemed to be quite normal, though, judging from the condition of specimens containing live parasites of the same size, they must have been originally in a fairly degenerate and exhausted condition. It may be noted that in the period immediately following the deposition of the eggs, the ovaries present a somewhat similar appearance to those of unparasitised larvae, being thin, flaccid and transparent, but gradually fill up anew with ovules, loaded with fat globules.

It seems, therefore, that in woodlice, as in many other Crustacea infested by parasites of various kinds, the effect of the parasite on the internal reproductive organs is primarily dependent on its large absorption of fat; that it causes degeneration of the female gonads because it not only diverts the supply of fat in the blood from the gonads to itself, but also withdraws the fat already stored up in the ovules; and that it has no perceptible effect on the male gonads, simply because these contain practically no fat, the spermatozoa, as is well known, being composed almost entirely of protein material.

No abnormalities in the external form of parasitised females have so far been observed. These individuals seem to be indistinguishable from normal

specimens in the pre-incubation stage. All those of which the oviducts were examined during the winter, were found to have been fertilised and to have masses of spermatozoa in the inner ends of the oviducts, so the presence of the parasite evidently does not prevent fertilisation. Parasitised females do, however, differ in a very important way from normal females. They never develop the brood pouch, and none studied up to the present exhibit either oostegites or cotyledons, even in a rudimentary form. They retain the immature form up to the moment when they are killed by the parasites. The abstraction of the fat material by the parasite is thus correlated with the arrest of development of the rudiments of both oostegites and cotyledons and the suppression of the moult at which these organs appear.

The study of the effect produced by parasites thus supports the statement made by Vandel and others, that the development of the oostegites and cotyledons is in some way connected with the maturation of the eggs in the ovaries; since the evolutionary cycle of the oostegites appears to correspond exactly with the evolutionary cycle of the ovary. Schöbl once asserted (1880, cited by Vandel, *loc. cit.*) that the development of the oostegites depends on fertilisation; but Verhoeff (1920), working with virgin females of *Oniscus murarius*, found that the oostegites develop normally independent of fertilisation. Vandel confirmed and extended these results with species of the genus *Trichioniscus*. In the centre and east of France *Trichioniscus provisorius* reproduces parthenogenetically; the brood pouch, nevertheless, develops in the usual way. Furthermore, Vandel was able to show that in the non-parthenogenetic form of *provisorius* inhabiting southern France, and in the allied, non-parthenogenetic *T. biformatus*, virgin females may produce the brood pouch, into which they deposit unfertilised eggs, which eventually degenerate.

Some further light has been thrown on the subject by the experiments of Mme M.-L. Le Roux (1931) on the amphipod crustacean, *Gammarus pulex*. Mme Le Roux observed that in these Crustacea certain hairs which appear on the oostegites in sexually mature females were absent in females infested by the larvae of the Acanthocephaline worm, *Polymorphus minutus*, which also produced in the host an arrest of development of the ovaries, so that they resembled those of females just after oviposition (cf. *supra*). In order to decide whether the parasite exerted a direct and independent effect on the setae and the ovaries or an indirect effect on the setae by arresting the development of the gonads, she castrated females by exposing them to the emanations of radium bromide during the period immediately following the moults. She found that in the irradiated specimens, vitellogenesis was eventually arrested and that in the later stages the oostegites lost the ovigerous setae. The oostegites themselves were not affected. This author concludes that, in female Amphipods at least, some of the secondary sexual characters are dependent for their appearance on the development of the gonads, while others (oostegites) develop independently. De La Vaulx (1921) has also maintained that the ephippium in the Cladocera is dependent for its development on a hormone secreted by the gonads.

These results harmonise very well with those obtained with parasitised woodlice. It may be of interest to note that in these also, Acanthocephaline larvae as well as Trematode Cercaria, and an undetermined Sporozoan parasite, have been found in over-wintering females of *Porcellio*, that in the infested individuals ovarian degeneration was always evident and that none of them were ever found to have developed the marsupium.

Nevertheless, though there certainly seems to be, in the females of these species, some relation between the development of the ovary and the development of the secondary sexual characters, it is not easy to understand what the relation can be. The only objective difference between the ovaries of normal and parasitised specimens appears to be in the accumulation of fat globules in the ovules, and it is not clear why the absence of these fat globules, which are generally considered to be merely passive reserve material, should affect the production of hormones by the gonad. It seems rather more simple to suppose that the parasite exerts its action by affecting the general metabolism, producing simultaneously the arrest of development of the rudiments of oostegites and cotyledons, and the disappearance of fat from the ovary. The experiments on *Gammarus* with radium emanations do not definitely disprove this, for it is possible that these emanations may act directly on the rudiments of certain of the secondary sexual organs, since the cells composing such rudiments often differ notably from those of the rest of the body.

(3) *The phagocytic reaction of the host*

As is well known, the parasites of Arthropods are often found to be more or less completely surrounded by envelopes made up of the white blood cells or phagocytes of the host. Many authors, following Metchnikoff (1892), still consider this to be a defensive mechanism. In some cases, parasite larvae are certainly surrounded by phagocytes while they are still living. Prof. E. H. Strickland (1930) found that this occurred frequently with the larvae of the Tachinid, *Gonia*, in Noctuid caterpillars. This Tachinid deposits its eggs on vegetation. They are swallowed by the caterpillar and hatch in the mid-intestine, producing minute maggots which bore through the intestinal wall into the body cavity, after which they migrate to the supra-oesophageal ganglion where they continue their development. Many of the larvae do not, however, reach the ganglion, but become surrounded by a dense envelope of phagocytes, in which they can be seen moving, but from which they seem unable to escape, and within which they invariably succumb after a few days. Data obtained by Prof. Strickland suggest that the embryos of *Meteorus vulgaris* Cress., developing in Noctuid caterpillars, may also be surrounded by phagocytes while still alive.

Cases of this kind are, however, exceptional. Generally speaking, the metazoan parasites of Arthropods, at least, are not, as a rule, surrounded by phagocytes while they are still living, unless they are attached to or partially embedded in some organ of the host, in which their presence has caused a

definite lesion. The exudate from lesions appears to cause the accumulation of phagocytes and consequently the envelopment of the parasite attached to the point of injury; but the envelopment is not usually complete and seems, in most cases, to have no detrimental effect on the parasite.

As has already been said, the larvae of dipterous parasites of woodlice appear to pass the greater part of their existence in an "integumental sheath," formed by an invagination of the cuticula and epithelium of the body wall around the posterior extremity of the parasite, which has thus, through its posterior spiracles and the opening of the sheath, access to the external air. Parasite larvae in integumental sheaths in insect hosts are not infrequently coated or surrounded with phagocytes, probably because of the attractive effect exerted on the blood cells by the exudate from the lesion, and such accumulations of phagocytes commonly occur around the larvae of the parasites of woodlice. The phagocytic layer varies enormously in extension and in thickness in individual cases. On the whole, however, larvae infesting *Oniscus asellus* seem to be more frequently and more thickly coated with phagocytes than those infesting *Porcellio scaber*. Parasites in individuals of *Oniscus* are sometimes completely enveloped in phagocytic sheaths. Such larvae have often a rather unhealthy appearance and may even be dead, especially toward the end of the winter.

If we hold the view that the function of the blood cells is to attack and destroy invaders we may be led to believe that only those parasites can survive that have developed mechanisms or substances able to repel their phagocytic enemies, and that the development of the specific mechanisms or substances is an important—perhaps the most important—aspect of the adaptation of an internal parasite to its host. On this view, the relative frequency of phagocytic accumulations around larvae in *Oniscus* is indicative of an inability to repel the phagocytes of the host.

The present writer is inclined to think that this view is fallacious, that phagocytes do not in actual fact possess any inherent disposition to attack invaders, and that the latter, if in a healthy condition, do not require any antiphagocytic substances to save them from a phagocytic onslaught, any more than do the tissues of the body of the host itself. It would be perhaps rash to suggest that the accumulation of the phagocytes is a purely passive phenomenon. It seems to be a fact that they exhibit, on occasion, change of shape and amoeboid movement, as when they penetrate into the degenerating muscle fibres and other tissues during the process of histolysis. But the behaviour of the phagocytes in relation to the tissue of the body during metamorphosis when compared with their behaviour in relation to normal tissues, very strongly suggests that their movements and their agglomerations are merely reactions to the presence of certain substances resulting from the degeneration of cells and tissues and depends primarily on changes in surface tension produced by pathological exudates.

If this explanation is valid, the accumulation of phagocytes around a

parasite indicates either that it is attached to a tissue in pathological condition or is itself in an unhealthy state. It is possible that during the developmental process certain eggs normally produce substances that attract or immobilise the phagocytes. On the other hand, the encystment of the larvae of *Gonia* may be due to the fact that these larvae have been covered with the secretions and contents of the intestinal cells in their passage through the gut to the body cavity.

It is possible, and even probable, that the phagocytes of different animals differ considerably in sensibility and that this is why the parasite larvae are more frequently encysted in *Oniscus* than in *Porcellio*. It seems, however, more probable that *Oniscus* is a less suitable host than *Porcellio*, not so much because of a difference in the composition of the cells and body fluids but because *Oniscus* habitually frequents damper places than *Porcellio* and is not infrequently partly immersed in moisture which closes the entrance to the integumental funnel and impedes the respiration of the parasite, producing a pathological condition which renders the larva a centre of attraction for phagocytes. The phagocytes agglomerate to form a rather thick and tenacious connective tissue, which, in some cases, covers the head of the larva, probably interferes seriously with its nutrition and causes the death of individuals that might otherwise be able to complete their development.

(4) *Hyperparasites*

In the spring of 1917 an Ichneumonid parasite belonging to the subfamily Cryptinae was bred from a puparium, found in the body of a *Porcellio*, collected in the cemetery of Haslar Hospital. This specimen was sent for determination to the Abbé Kieffer in 1920, but no report was ever received from him. In 1926, however, Mr O. W. Richards bred what seems to be a similar species from a dead woodlouse, collected under a stone at Hinksey, Berks., near Oxford, on May 15th. This specimen, which is a female, has been determined by Dr C. Ferrière as *Phygadeuon vexator* Thunb. (*-dumetorum* Grav.). Further details in regard to this parasite will be given in a later paper. It is, however, interesting to note that *Phygadeuon vexator* has been reared from several Tachinids and Ichneumonids, parasitic on Lepidoptera, and that the development and pupation of the Tachinid in a Crustacean has not fundamentally altered the affinity.

VI. The morphological adaptation in the parasites of woodlice

It is very generally admitted by biologists, that parasitic organisms present some of the best examples of morphological adaptation, or, in other words, of the functional suitability of the bodily structures to environmental conditions. Entomophagous insects are, of course, for the most part, parasitic only in certain stages of the life history. The adult forms are free-living; ordinarily, only the larval stages are parasitic in the true sense. Since the adults of the species parasitic on woodlice are free-living organisms they cannot be expected

to exhibit any morphological adaptations to a parasitic life. They might, however, present certain structural mechanisms or structures useful, or apparently of use, in introducing their eggs or larvae into the bodies of the woodlice, or into the colonies of these animals, comparable, for example, to the ovipositors of the Hymenoptera parasitica.

As a matter of fact, however, the dipterous parasites of woodlice have, in the adult stage, practically no common structural characters that are not found in the other members of the group of Rhinophorine Tachinids. According to Villeneuve (1924) these flies all have their antennae inserted below the middle of the eye; the frontal setae extending only to the base of the antennae; the thorax bearing ordinarily only the median pair of presutural acrostichal bristles, or none; the scutellum with two or three marginal setae of equal length; the first abdominal segment elongate, feebly excavated; the abdominal setae rather feeble; the abdominal sternites more or less uncovered in the males but generally hidden in the females; the first posterior cell of the wing frequently petiolate, or open at the wing tip; the costal spine usually prominent; the thoracic alulet small. Many species have the parafacials ciliate or provided with prominent bristles. Frequently the palpi are notably abbreviated; the mouth is often protuberant, or the clypeus prolonged below; the ascending frontal setae are generally absent.

It seems impossible to discern, in these characteristics, anything having a positive relation to the habit of attacking woodlice or even to the parasitic habit in any sense of the term. So far as one can see, they have in themselves nothing but a purely taxonomic significance and have absolutely no formal correlation with the method of reproduction or the mode of life of the larvae. The morphological characteristics of the genera and species of these parasites appear to be equally void of adaptive significance. As is pointed out in another part of this paper, almost all the species here studied attack *Porcellio scaber*, though some have been found also to infest other woodlice, such as *Armadillidium*, *Oniscus*, and *Metaponorthus*. All may therefore be considered to be adapted to attack the same host. Furthermore, though striking morphological differences exist between the adults of these various genera and species, the reproductive habit and the mode of attack appear to be the same in all of them, so that it is impossible to see any formal correlation between the specific characters of the various forms and the method of bringing the offspring into contact with the host. The ovipositor of the female is of the type normally found in the non-parasitic Muscids, such as the house-fly, and does not exhibit in any of the species so far studied any modifications that can be correlated with the parasitic habit.

Furthermore, not only does it seem impossible to discover any real correlation between the morphological characters of the several species of this group, or the characters distinguishing the group as a whole, and the habit of attacking woodlice, but it is very difficult to attribute to those structural features *any* adaptive significance whatever. All or most of the species may be found in the

adult stage in the same type of environment, so it is evident that, considered in respect of life in that environment, the characters by which they differ can have no vital importance; but in any event, it is extremely difficult to see how such features as the form of the first posterior cell of the wing, the pilosity or nudity of the arista, the precise disposition of the macrochaetae on the head, thorax, and abdomen, can either aid or impede the existence of these parasites to any noticeable degree. In other words, there is, in fact, no reason to believe that such characters have what is called "survival value." They are, however, what the taxonomist ordinarily calls "specific characters." It follows that in so far as these organisms are concerned, the "specific characters" can hardly have been produced by any selective process.

Generally speaking, this conclusion seems to be valid for the parasitic Muscids in general. The numerous species of this group exhibit, on the whole, a fairly considerable structural diversity; but it is practically impossible to assign to the specific characters any precise functional significance, or to assert in any given case that the specific conformation has in itself any precise adaptive significance. All of these forms reproduce and survive in the environments where we find them. We must therefore admit that they are in a very real sense adapted to these environments; but it is apparently not in the characters by which we distinguish the various species, genera, tribes, and subfamilies, that the adaptive virtue has its seat.

We arrive at this same conclusion when we consider the larval anatomy of the parasites of woodlice. The available data indicate that all of these species deposit undeveloped eggs in the vicinity of the colonies of woodlice and that the primary larvae make an active search for their hosts. Nevertheless, the primary larvae of some species, such as *Cyrillia angustifrons*, *Frauenfeldia rubricosa*, and *Plesina maculata*, possess a strongly developed cuticular armour, while those of other species, such as *Melanophora roralis* and *Styloneuria discrepans*, seem to be devoid of protective cuticular structures. Furthermore, although the buccopharyngeal armature of the first-stage larva is of the same type, with an unusually elongate intermediate region and an articulation at the base of the anterior tooth, the differences in form of the various parts, and especially of the anterior teeth, are considerable. Nevertheless, practically all of these larvae are found in individuals of *Porcellio scaber* and therefore, in spite of their morphological diversity, all of the mouth-hooks of the parasites attacking this host may be considered to be equally well adapted for the work of making an opening through its body wall.

Again, considerable differences exist between the buccopharyngeal armatures of the second- and third-stage larvae of the various parasites of *Porcellio scaber*, as well as between the anterior spiracles; but all of these larvae appear to live in the same way, in exactly the same environment, and consequently it is very difficult to see how these specific morphological characteristics can have any adaptive significance whatever. So far as we can see, all of these different tools serve equally well for the same purpose. One might

indeed on *a priori* grounds consider one form of mouth-hook a more effective penetrating instrument than another; but it would be practically impossible to test the hypothesis.

We have then no reason to maintain that the specific morphological conformations of any of the various dipterous parasites of woodlice are really adaptive in the sense that this one particular conformation, and this alone, adequately meets the needs of the situation. These specific characteristics, as we have already seen them, cannot therefore have any "survival value" and cannot have arisen as the result of a selective process, though it is the description of these characters that contributes the taxonomic definition of the species.

It is, of course, possible to reply that if we had an absolutely thorough knowledge of the life of an organism in its minutest details, the significance of its structure—the definition and positive usefulness of all its morphological characteristics—would be clearly apparent to us. But it seems equally probable that the long continued and persistent attempts to interpret in purely utilitarian terms the endless variations in organic structure, derives on the one hand from a preconceived idea as to the origin of specific characters and, on the other hand, from an inadequate view of the nature of adaptation. According to this view of adaptation it is essentially a kind of *material and static conformity between things*. The organism is considered to be adapted to its environment in much the same way as the outline of a lake is adapted to the undulations in the line of the shore, or as the surface of an irregular boulder is adapted to the earth in which it is embedded. Through his prolonged and intensive study of structure, the morphologist has been almost inevitably led to consider their geometrical form as the most important feature of living things. He therefore tends naturally to imagine that function is strictly and intrinsically dependent on form, and that the purpose of an organ can be scientifically deduced from its structure. The analogy between organisms and the mechanisms devised by man for various purposes and between the several parts of organisms and man's common tools, in which the correspondence between structure and function seems to be self evident, appears to provide a very solid support for the view of adaptation as something fundamentally morphological.

Nevertheless, though tools have very great biological significance, being, in a certain sense, as I think Samuel Butler pointed out somewhere, a prolongation or extension of the organism that uses them, they do not really exemplify or demonstrate the dependence of function on structure. Tools indeed represent the materialisation of a *purpose*—of an *intention*. But intentions and purposes are ideas, belonging to the world of pure forms in the Platonic sense; and no material thing can completely exhaust their content. Thus, such a simple purpose as the breaking up of a block of marble can be accomplished practically in a dozen different ways and by the use of a number of radically different instruments. On the other hand, since a tool really exists as such during the period when it is held and manipulated by the user and derives its significance as an instrument only from the purpose for which it is used, it is evident that

the same tool may serve many different purposes or, to put the point more accurately, the same material object may become, in the hands of different users, or on different occasions, a multitude of different tools.

Exactly the same thing is true of the organs of the living being. Take, for example, the mandible of the grasshopper: such an object may be considered in two very different ways. Taken by itself, apart from the organism to which it happens to belong, it is merely a mass of chitinised pigmented material, having certain quantitative, physical, and chemical characteristics. This is absolutely all that we can say about it from this standpoint. But we can also consider it as forming part of a certain living being, which uses it in a certain way for certain purposes, such as the mastication of its food. We then call it a mandible; but considered apart from the living being, without relation to the purpose for which the living being uses it, it is, strictly speaking, an amorphous, and so far as biological concepts are concerned, an indefinable and nameless object.

It would, of course, be rather unreasonable to say that there is absolutely *no* relation between the form of an organ and the purpose for which it is used, but the relation is of an extremely vague and general order and practically eludes any attempt at definition. In a word, the relation between the form and the function of an organ is a contingent relation; the use made of the organ depends not so much on its shape and structure as on the general characteristics of the creature of which it is part. Organs almost identical in form may have markedly different functions in different organisms, while organs very dissimilar in structure may serve in different organisms for the same purpose.

Since function is not primarily dependent on form, it follows that adaptation in the sense of the term is not fundamentally a *morphological* or *structural state*. The true test of the adaptation of an organism to its environment is the ability to live in that environment. If an organism can live under certain conditions, it is adapted to those conditions, whether it presents anything that we can interpret as a morphological conformity with the conditions or not. Adaptation is in fact simply another name for the immanent movement we call life. The examination of a series of carefully selected examples may suggest an extremely close and necessary correlation between organic form and environmental conditions; but an intensive study of the systematic allies of any one of these examples will rapidly dissipate the illusion.

VII. THE HOST RELATIONS OF THE PARASITES OF WOODLICE

During the course of these studies, specimens of *Ligia oceanica*, *Oniscus asellus*, *Philoscia muscorum*, *Porcellio scaber*, *P. dilatatus*, *Metaponorthus pruinosus*, *Cylisticus convexus*, and *Armadillidium vulgare* have been dissected; but parasites have been found only in *Porcellio scaber*, *Oniscus asellus*, *Metaponorthus pruinosus*, and *Armadillidium vulgare*. Parasitised specimens of *Metaponorthus pruinosus* have been found only in south-western France, where

this species is attacked by *Cyrillia angustifrons*. No dipterous parasites have been found in any of the specimens of *Armadillidium vulgare* dissected, but from an individual found by Donisthorpe (1908) in the Isle of Wight, an adult of *Phyto melanocephala* was reared. *Oniscus asellus* was found at Haslar (Hants.) to be parasitised occasionally by *Styloneuria discrepans* and *Plesina maculata*. *Porcellio scaber* is attacked by *Plesina maculata*, *Phyto melanocephala*, *Melanophora roralis*, *Styloneuria discrepans*, *Frauenfeldia rubricosa*, and the undetermined species with the double mouth-hook.

The results appear to indicate that *Porcellio scaber* is, on the whole, the favoured host of these parasites. *Cyrillia angustifrons* was found only in *Metaponorthus pruinosus*; but in the region where this extremely rare Tachinid was discovered, no colonies of *Porcellio scaber* or *Oniscus asellus* were observed so that the parasitism of *Cyrillia* in *Metaponorthus* is not necessarily specific. It is also clear that not all the parasites found in *Porcellio scaber* are specific enemies of this Isopod, since *Phyto melanocephala* has been obtained from *Armadillidium vulgare*, while *Plesina maculata* and *Styloneuria discrepans* also attack *Oniscus asellus*. The distribution of some species is evidently determined to some extent by meteorological conditions. Thus, although *Styloneuria discrepans* is occasionally found at Farnham Royal, it is much more abundant in districts with a maritime climate (Haslar, Isle of Wight, Looe). That most of the parasites are incapable of attacking successfully woodlice belonging to species other than those in which they are commonly found, seems somewhat unlikely. It is of interest in this connection to note that certain quite different parasites of these Isopods are not particularly specific in their host relations. Thus the *Cercaria* of what seems to be the same species of Trematode were found in both *Oniscus asellus* and *Porcellio scaber*; a Coccidian was observed in *Porcellio scaber*, *Oniscus asellus*, and *Philoscia muscorum*; while Echinorhynchus larvae, considered by my friend, Dr H. A. Baylis, to be specifically identical, occur in *Porcellio scaber*, *Armadillidium vulgare*, and *Philoscia muscorum*.

These facts suggest that the dipterous parasites of woodlice could develop in individuals of almost any terrestrial Isopod and that the prevalence of most species in *Porcellio scaber* and *Oniscus asellus* is due principally to the fact that these species occur much more commonly than the others in large colonies under the loose bark of fallen logs; that is to say, under conditions favouring the attack of the dipterous parasites. The other species of *Porcellio* and *Oniscus*, as well as those of the genus *Metaponorthus*, are, in general, less common. *Philoscia muscorum* is an active and wandering species, living amongst grass; *Cylisticus* and *Armadillidium* do not usually occur under bark and have a strongly arched body with the ventral surface some distance above the ground, and are, moreover, very heavily armoured, so that it may be somewhat difficult for the parasite larva to effect an entrance.

On the other hand, there are some indications that the specific constitutional properties of the host are not without importance. The larvae of

Plesina maculata and *Styloneuria discrepans* found in *Oniscus asellus* are in general more frequently surrounded by phagocytic envelopes than those found in *Porcellio scaber*. On one occasion a larva of the undetermined species was removed from the original host (*Porcellio scaber*) and inserted through an artificial opening into the body of a specimen of *Philoscia muscorum*. It was still living four days after transplantation; but twelve days later it was found to have died. It may be noted that larvae transplanted to a Harpaline Carabid, to *Galerucella luteola*, and to *Lithobius forficatus* also died or showed signs of ill-health, though the specimen placed in the Carabid was definitely alive seven days after its transference to this host.

Nevertheless, the data available at the present time appear, on the whole, to suggest that the distribution of the parasites of woodlice in the series of possible hosts depends, in the main, on the habits of the latter, and that any of the known species might be a satisfactory host if it lived in large colonies under the loose bark of logs and fallen trees, as does *Porcellio scaber*. It is true that although *Oniscus asellus* is sometimes found in such situations, only 3·1 per cent. of all specimens of this species dissected were found to be parasitised as against 9·2 per cent. of *Porcellio scaber*. The fact that *Oniscus asellus* is less frequently parasitised than *Porcellio scaber* is, however, not necessarily due to the intrinsic unsuitability of the former as a host. It more probably depends on the difference in the habit of the two species. *Oniscus asellus*, as we have already noted, requires more moisture than *Porcellio scaber* and lives in damper places and is consequently more difficult of access to parasites with the reproductive habits of the Rhinophorine flies.

VIII. THE NATURAL CONTROL OF WOODLICE

Parasitic insects are often considered to be by far the most efficient natural regulatory agents in relation to a very large number of the hosts on which they prey. If this view is justified one would expect the insect parasites of woodlice to be of considerable importance in regulating the numbers of these organisms; but the data at present available do not at present suggest this. Certain species, such as *Porcellio laevis*, *Cylisticus convexus*, and *Philoscia muscorum*, do not appear to be attacked by the Tachinids, while others, such as *Armadillidium vulgare* and *Metaponorthus pruinosus*, are very rarely parasitised, at least in most districts. The table on p. 442 gives the results of dissections made during the work. The average parasitism is relatively low. Of 1737 specimens of *Porcellio scaber* and *Oniscus asellus*, collected in some eighteen different localities in France and England, and dissected during 1917, 1919, 1929, and 1931, only 9·1 per cent. were parasitised. The average parasitism of *O. asellus* was only 3·1 per cent. The highest parasitism noted in lots sufficiently large to estimate percentages with any degree of accuracy (100 or over) was 7·2 per cent. for *O. asellus* at Haslar, Hants. (1917) and 25·2 per cent. for *Porcellio*

scaber at Farnham Royal, Bucks. (1931). These figures indicate in every case the combined parasitism by all the species of Tachinids found in the dissections of material from the localities mentioned, though of course the relative importance of the various species differ in different hosts, localities and seasons:

		Porcellio scaber		*Oniscus asellus*		*Metaponorthus pruinosus*		*Porcellio laevis*	
Locality	Year	(1)*	(2)*	(1)	(2)	(1)	(2)	(1)	(2)
Haslar, England	1917	311	27	151	11	—	—	—	—
Ornezan, Gers, France	1919	—	—	—	—	16	1	—	—
Bois de Verrières, France	1919	8	1	—	—	—	—	—	—
Paris, France	1919	14	0	—	—	—	—	—	—
St Cloud, France	1919	6	1	13	0	—	—	—	—
New Forest, England	1919	84	17	52	0	—	—	—	—
Rowland's Castle, Hants., England	1919	—	—	12	0	—	—	—	—
Seine-et-Oise, France	1919	20	2	20	0	—	—	—	—
Elbeuf, France	1929	264	6	—	—	24	0	—	—
Princes Risborough, England	1929	42	0	—	—	—	—	—	—
Farnham Royal, England	1929	270	68	12	0	60	0	—	—
Looe, England	1931	20	8	—	—	—	—	—	—
Isle of Wight, England	1931	—	—	20	0	—	—	—	—
Hastings, England	1931	58	1	—	—	—	—	—	—
Loughton, England	1931	50	1	50	0	—	—	—	—
Didcot, England	1931	23	1	—	—	—	—	—	—
Sandown, England	1931	40	5	—	—	—	—	—	—
Farnham Royal, England	1931	80	0	17	0	—	—	—	—
Antibes, France	1931	—	—	—	—	—	—	76	0
		1290	138	347	11	100	1	76	0
		10·7 %		3·1 %		1·0 %		0·0 %	

* (1) Examined; (2) Parasitised.

Species	Examined	Parasitised
Porcellio scaber	1290	138
Oniscus asellus	347	11
Metaponorthus pruinosus	100	1
	1737	150
	9·2 % approx.	

It is thus evident that the dipterous parasites are not factors of major importance in the control of woodlice. Nevertheless, since under certain conditions as many as a quarter of the host population succumbs to the parasitic attack, it seems, at first sight, that the Tachinids must play a very real part in control.

It must, however, be noted that the effect exerted by the parasite on the male woodlice is apparently negligible, so far as control is concerned. No infested females appear to lay eggs; but there is no indication that the presence of the parasite interferes with the development of the male reproductive organs or inhibits copulation, which apparently takes place in the late summer or autumn, while the parasite larva is still rather small. It seems that in some cases it kills the woodlouse in the spring following its entry, whereas the life of unparasitised specimens, both male and female, possibly extends, under favourable conditions, through several years, but some larvae appear to require more than one season for their development.

Furthermore, although the total absence of the parasites in any particular locality would certainly permit of the production of a greater total number of offspring, it is difficult to believe that the ultimate effect would be very noticeable. The number of unparasitised females coming to maturity in any given season is always considerable and, in a colony of average size, sufficient to increase the population enormously, even in the course of a single generation, since the number of offspring is large. It is safe to say that if all the young produced in the average colony of *P. scaber* actually survived, the food supply would be very rapidly exhausted and the majority of the members of a colony would be obliged to migrate, or would practically all die of starvation, even if the space available under the bark of the average log were sufficient to contain them.

It is fairly obvious that colonies do not generally increase at such a rate. There must therefore be, under average conditions, a very high mortality which is probably greatest among the very young specimens and is due partly to the habit of cannibalism, which is very common among woodlice, individuals rendered defenceless by the moult being frequently devoured by their companions.

The fact is, that although isolated specimens of such species as *Porcellio scaber* are found in a variety of situations, conditions suitable for the establishment of one of the colonies in which they normally live, seem to be rather narrowly circumscribed and, in the more thickly populated regions, rather uncommon. In any given environment of this type, the normal rate of increase very soon produces overcrowding, and as a result of this the population tends to numerical stability. To the elimination of individuals that necessarily takes place under these conditions, the parasites contribute to a greater or less extent; but whether they are present or not, the increase in numbers must inevitably be arrested sooner or later. Furthermore, if as suggested here, the natural control of these animals is effected chiefly by a progressive rise in the death rate in the early stages, many parasites will die with their hosts, and although an increase in population density ought to be favourable to larvae obliged to go in search of their prey, this advantage will be largely offset by the rise in the general death rate of the young woodlice in crowded conditions.

Parasites are not therefore necessary for the control of such gregarious woodlice as *Porcellio scaber*, *Metaponorthus pruinosus*, and *Oniscus asellus*. Against solitary or wandering species, forms with habits like those of the Rhinophorines would apparently be even less effective. Nevertheless, though woodlice of this type are seldom if ever attacked, they are not noticeably more abundant than the gregarious woodlice. Parasites are certainly not essential for the natural control of these forms and there is no particular reason why we should consider them as essential for the control of any of the other species of the group.

It is, of course, quite conceivable that the dipterous parasites might by themselves be capable of controlling the numbers of woodlice. The fundamental requisite for this is that the parasites should become capable of reproducing at

the same rate as the woodlice, whenever the latter begin to increase in numbers or whenever the population density of the woodlice exceeds a certain point. If under normal conditions no other environmental factors were able to check the increase in numbers until long after the parasites were able to arrest it, then the population level, in the presence of parasites, would tend always to be definitely lower than it would in their absence.

Generally speaking, however, the dipterous parasites of woodlice seem to be incapable of increasing at the expense of their hosts before other regulatory factors, such as starvation and cannibalism, have already come into play. They appear, in fact, to be extremely inefficient as regulatory agencies. The colonies of woodlice usually live under logs, bark, or stones. They are able to live and move about in spaces too exiguous for the entrance of the adult parasites. Under these circumstances, the habit of depositing the eggs in the vicinity of the colonies of the host gives the parasite larvae some chance of finding a host, but it is easy to see that the probability of the survival of an individual larva is exceedingly small, and that it is considerably decreased by the long developmental period intervening between oviposition and the emergence of the larva. In spite of the inefficient and haphazard reproductive habit, the number of offspring produced is not very large. Certain of the Echinomyiine flies, which deposit larvae in the vicinity of the caterpillars they attack, produce many thousands of offspring, while the Rhinophorines produce at most only a few hundred. The extraordinary fecundity of the Echinomyiines is, of course, no advantage to the individual, in no way increases its chance of survival, and is not, in any real sense, an adaptation. It does, however, enhance the value of the parasite as a controlling agency, enabling it to attain and destroy individuals of the host, even in small colonies, while the low reproductive power of the Rhinophorines must render them very inefficient unless the host is very abundant.

There is, therefore, little reason to think that the dipterous parasites of woodlice are necessary or even important factors in the natural control of their hosts. It is possible that under other conditions, more favourable to their peculiar and inefficient habits, they might be able to dominate their hosts and bring them under control before the non-parasitic factors come into action; but there is at present no indication that they are capable of such action.

The proportion of woodlice destroyed by the dipterous parasites varies considerably from place to place and from year to year. The reason for these fluctuations is at present unknown. An indefinite number of departures from the optimum conditions could produce the same numerical result. Meteorological conditions at the reproductive period are probably of great importance. In 1917, at Haslar, some 90–95 per cent. of the female woodlice bore developing embryos during the latter part of May. On June 1st, puparia of the parasites were to be found in the field. Now unless the hatching of the eggs deposited by the flies coincides with the appearance of an abundance of young woodlice in a stage suitable for parasitisation, a great many of the offspring of the parasites

will be lost. A difference in the physiological activity of host and parasite under different conditions of humidity, rainfall, and temperature might therefore be of great importance.

Furthermore, the irregular distribution in time and space of habitats suitable for the gregarious woodlice, inevitably restricts the multiplication of the parasites. Logs that have been left lying on the ground until the bark has become detached from the wood seem to offer the optimum habitat for the species most commonly parasitised. But as has already been said, in thickly settled countries, logs are not often allowed to lie about until this stage, and since those in favourable condition are often rather widely separated, it may be some time before they are invaded and several years before they harbour a large colony. In other words, of all the rather scattered habitats existing at any given moment, not all will contain colonies. The adult parasites, being highly mobile creatures, will tend to spread outward from the colony as soon as they are able to fly, and it is possible that many of them will never again discover it or any other colony containing hosts sufficiently accessible or in sufficient numbers. Finally, although individual woodlice or small groups of woodlice can subsist apart from the habitats suitable for colonies and are, in fact, always to be found scattered about everywhere throughout the environment, such essentially mobile specimens are practically inaccessible to the parasite.

IX. Summary

1. This paper comprises a study of the larval morphology and biology of the dipterous parasites of the terrestrial Isopods or woodlice.

2. *Porcellio scaber* L., *Oniscus asellus* L., *Metaponorthus pruinosus* Brandt., and *Armadillidium vulgare* Lat., were found to be attacked by one or more of the parasites.

3. Seven dipterous parasites have been reared and identified from woodlice: *Plesina maculata* Fall., *Melanophora roralis* L., *Phyto melanocephala* Meig., *Styloneuria discrepans* Pand., *Frauenfeldia rubricosa* Meig., *Cyrillia angustifrons* Rond., and *Rhinophora lepida* Meig. The biology and larval forms of all of these except *R. lepida* are described and figured, together with those of two undetermined species, "A" and "B."

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EXPLANATION OF PLATES XV–XXII

PLATE XV

Fig. 1. *Plesina maculata* Fall. Buccopharyngeal armature, Stage I. ×954.
Fig. 2. *Plesina maculata* Fall. Antenna, Stage I. ×1485.
Fig. 3. *Plesina maculata* Fall. Anterior sclerite, buccopharyngeal armature, Stage I. ×1485.
Fig. 4. *Plesina maculata* Fall. Anterior stigma, Stage III. ×207.
Fig. 5. *Plesina maculata* Fall. Larva, Stage I, dorsal view: *p.s.* posterior stigmata. ×207.
Fig. 6. *Plesina maculata* Fall. Posterior extremity, larva, Stage I, dorsal view: *p.s.* posterior stigmata. ×1440.
Fig. 7. *Plesina maculata* Fall. Posterior extremity, larva, Stage I, ventral view. ×1440.
Fig. 8. *Plesina maculata* Fall. Posterior trachea, felt-chamber and spiracle, Stage II. ×639.
Fig. 9. *Plesina maculata* Fall. Posterior stigmata, surface view. ×135.
Fig. 10. *Plesina maculata* Fall. Cuticular armature of larva, Stage I. ×1485.
Fig. 11. *Plesina maculata* Fall. Larva, Stage II, *in situ*, showing respiratory relation with skin of host (*P. scaber*): *o.s.* orifice of respiratory sheath.

PLATE XVI

Fig. 12. *Frauenfeldia rubricosa* Meig. Reproductive system, adult female: *a.g.* accessory gland; *ov.* ovary; *sp.* spermathecae.
Fig. 13. *Plesina maculata* Fall. Larva, Stage II, lateral view. ×30.
Fig. 14. *Frauenfeldia rubricosa* Meig. Reproductive system, adult male: *a.g.* accessory glands; *t.* testes.
Fig. 15. *Plesina maculata* Fall. Buccopharyngeal armature, Stage II, lateral view. ×639.
Fig. 16. *Plesina maculata* Fall. Internal spiracle, pupa. ×225.
Fig. 17. *Plesina maculata* Fall. Larva, Stage III, lateral view.
Fig. 18. *Plesina maculata* Fall. Buccopharyngeal armature, Stage III. ×285.
Fig. 19. *Styloneuria discrepans* Pand. Anterior and intermediate regions of buccopharyngeal armature, Stage III: *a.s.* anterior sclerite; *a.v.* anterior ventral band; *h.s.* hypopharyngeal sclerite; *i.s.* intermediate sclerite. ×315.

PLATE XVII

Fig. 20. *Styloneuria discrepans* Pand. Buccopharyngeal armature, Stage I. ×954.
Fig. 21. *Styloneuria discrepans* Pand. Posterior stigmata, Stage III, surface view. ×135.
Fig. 22. *Styloneuria discrepans* Pand. Internal spiracle, pupa, surface view. ×216.
Fig. 23. *Styloneuria discrepans* Pand. Buccopharyngeal armature, Stage II, lateral view. ×639.
Fig. 24. *Styloneuria discrepans* Pand. Anterior sclerite, buccopharyngeal armature, Stage I. ×1485.
Fig. 25. *Phyto melanocephala* Meig. Antenna, Stage I. ×297.
Fig. 26. *Styloneuria discrepans* Pand. Anterior stigma, Stage III. ×378.
Fig. 27. *Phyto melanocephala* Meig. Buccopharyngeal armature, Stage I. ×954.
Fig. 28. *Phyto melanocephala* Meig. Anterior sclerite, buccopharyngeal armature, Stage I. ×1485.
Fig. 29. *Styloneuria discrepans* Pand. Buccopharyngeal armature, Stage III. ×225.

PLATE XVIII

Fig. 30. *Styloneuria discrepans* Pand. Puparium, dorsal view.
Fig. 31. *Phyto melanocephala* Meig. Buccopharyngeal armature, Stage II. ×576.
Fig. 32. *Porcellio scaber* L., containing empty puparium of *Plesina maculata*, Fall., showing cap of puparium and opening through which fly emerged from host.
Fig. 33. *Phyto melanocephala* Meig. Buccopharyngeal armature, Stage III. ×225.
Fig. 34. *Phyto melanocephala* Meig. Internal spiracle, surface view. ×225.
Fig. 35. *Phyto melanocephala* Meig. Posterior end of puparium, showing stigmata.
Fig. 36. *Plesina maculata* Fall. Oesophageal caecum. ×190.
Fig. 37. *Phyto melanocephala* Meig. Posterior stigmata, surface view.

PLATE XIX

Fig. 38. *Melanophora roralis* L. Buccopharyngeal armature, Stage I. ×1485.

Fig. 39. *Melanophora roralis* L. Posterior extremity, puparium.

Fig. 40. *Melanophora roralis* L. Buccopharyngeal armature, Stage III. ×225.

Fig. 41. *Melanophora roralis* L. Anterior stigma, Stage III. ×378.

Fig. 42. *Melanophora roralis* L. Antenna, Stage I. ×1485.

Fig. 43. *Melanophora roralis* L. Posterior extremity, larva, Stage III, showing posterior tracheae, felt chambers, and the corresponding organs of Stage II, partially moulted: *t*2, tracheae of Stage II; *t*3, tracheae of Stage III.

Fig. 44. *Melanophora roralis* L. Buccopharyngeal armature, Stage II. ×639.

Fig. 45. *Melanophora roralis* L. Internal spiracle, pupa. ×639.

Fig. 46. *Cyrillia angustifrons* Rond. Anterior sclerite, buccopharyngeal armature, Stage I. ×1485.

Fig. 47. *Cyrillia angustifrons* Rond. Buccopharyngeal armature, Stage I. ×378.

PLATE XX

Fig. 48. *Cyrillia angustifrons* Rond. Buccopharyngeal armature, Stage II. ×570.

Fig. 49. *Cyrillia angustifrons* Rond. Anterior stigma, Stage III. ×639.

Fig. 50. *Cyrillia angustifrons* Rond. Antenna, Stage I. ×1485.

Fig. 51. *Frauenfeldia rubricosa* Meig. Buccopharyngeal armature, Stage I. ×1485.

Fig. 52. *Cyrillia angustifrons* Rond. Posterior stigmata, Stage III, surface view. ×225.

Fig. 53. *Cyrillia angustifrons* Rond. Cuticular protuberance, Stage I. ×144.

Fig. 54. *Frauenfeldia rubricosa* Meig. Posterior extremity of puparium.

Fig. 55. *Frauenfeldia rubricosa* Meig. Anterior stigma, Stage III. ×225.

Fig. 56. *Cyrillia angustifrons* Rond. Buccopharyngeal armature, Stage III. ×225.

Fig. 57. *Cyrillia angustifrons* Rond. Internal spiracle, pupa. ×378.

Fig. 58. *Frauenfeldia rubricosa* Meig. Posterior stigmata, surface view.

Fig. 59. *Frauenfeldia rubricosa* Meig. Anterior sclerite, buccopharyngeal armature, Stage I. ×378.

PLATE XXI

Fig. 60. *Frauenfeldia rubricosa* Meig. Buccopharyngeal armature, Stage II. ×576.

Fig. 61. *Frauenfeldia rubricosa* Meig. Internal spiracle, pupa.

Fig. 62. Species B. Anterior sclerite, buccopharyngeal armature, Stage I. ×1485.

Fig. 63. Species B. Antenna, Stage I. ×954.

Fig. 64. Species B. Anterior stigma, Stage III.

Fig. 65. *Frauenfeldia rubricosa* Meig. Buccopharyngeal armature, Stage III. ×252.

Fig. 66. *Frauenfeldia rubricosa* Meig. Digestive system, larva, Stage II: *a.m.t.* anterior Malpighian tubes; *m.i.* mid-intestine; *n.s.* nervous system; *oe.c.* oesophageal caecum; *oe.v.* oesophageal valve; *p.i.* posterior intestine; *p.m.t.* posterior Malpighian tubes; *s.g.* salivary glands.

Fig. 67. *Frauenfeldia rubricosa* Meig. Cuticular protuberance with a sensorium, Stage I.

Fig. 68. Species B. Buccopharyngeal armature, Stage I. ×378.

PLATE XXII

Figs. 69, 70. Species B. Cuticular organs, outer side. ×1485.

Figs. 71, 72. Species B. Cuticular organs, inner side. ×1485.

Fig. 73. Species A. Digestive system, larva, Stage I, cf. Fig. 66.

Fig. 74. Species A. Buccopharyngeal armature, Stage I, lateral view. ×639.

Fig. 75. Species A. Posterior extremity, larva, Stage I, dorsal view: *p.s.* posterior stigma; *s.* sensorium.

Fig. 76. Species A. Buccopharyngeal armature, Stage II. ×432.

Fig. 77. Species A. One of sensorial organs shown in Fig. 75, with smaller rod-shaped sensoria at its base.

Fig. 78. Species A. Anterior extremity of buccopharyngeal armature, Stage I, dorsal view. ×540.

Fig. 79. Species A. Cuticular armature, larva, Stage I. ×1485.

(*MS. received for publication* 1. II. 34.—Ed.)

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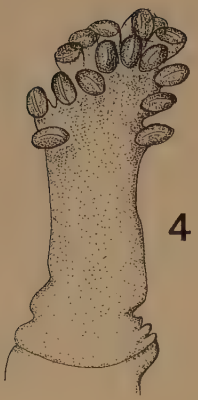

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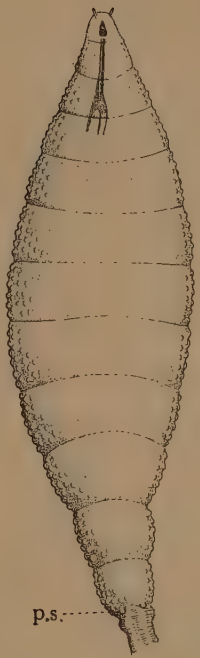

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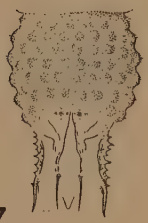

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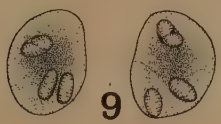

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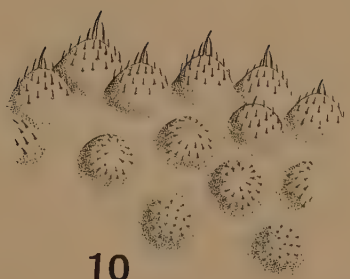

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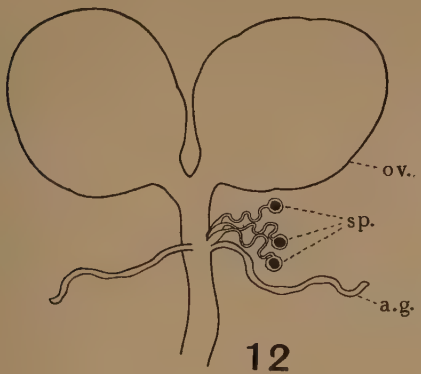

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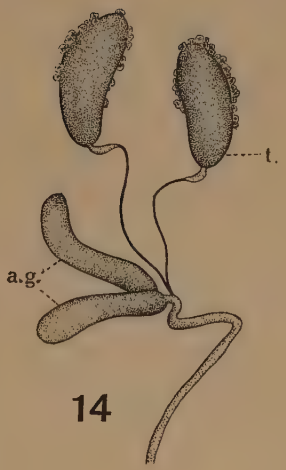

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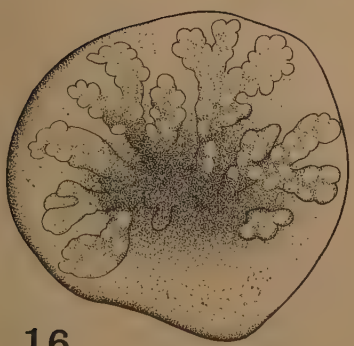

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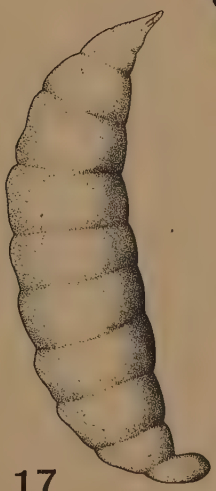

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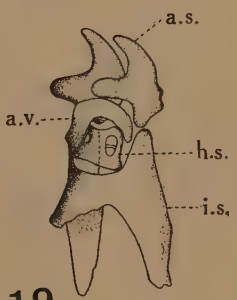

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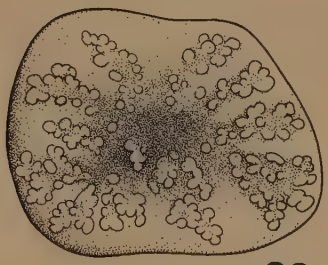

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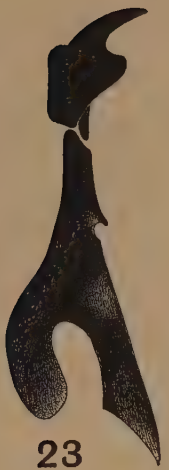

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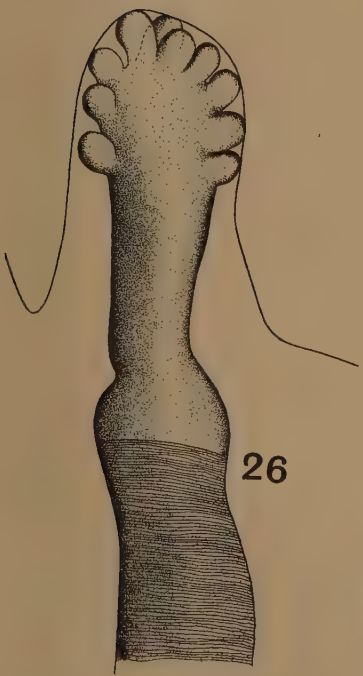

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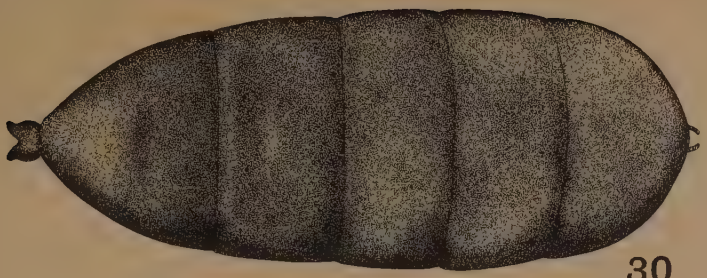

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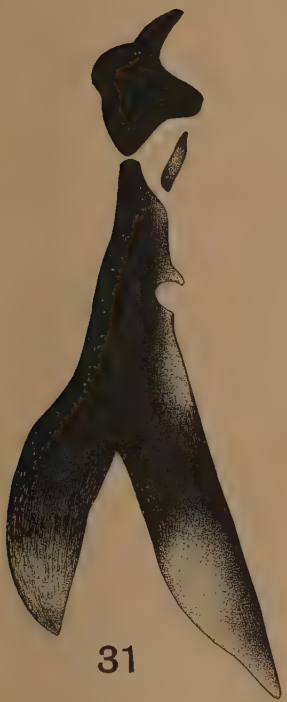

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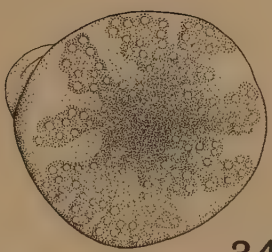

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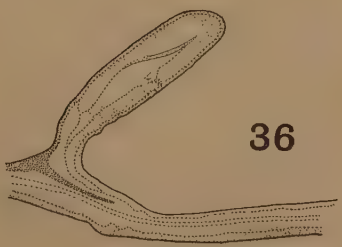

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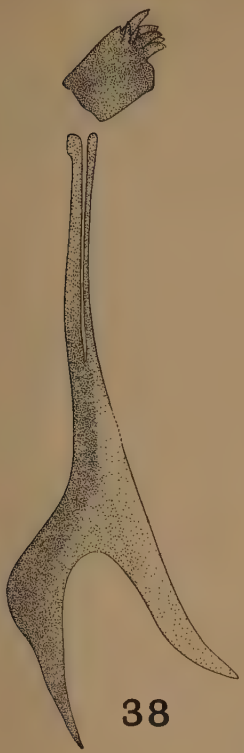
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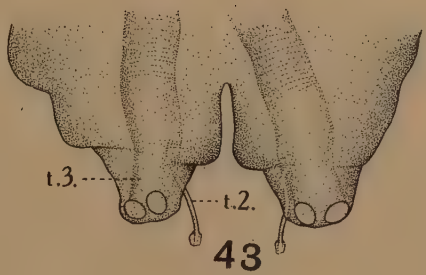

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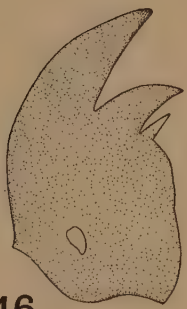
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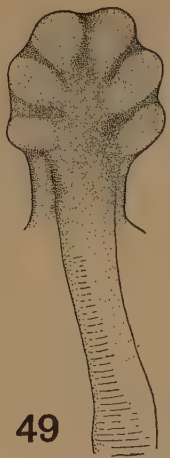
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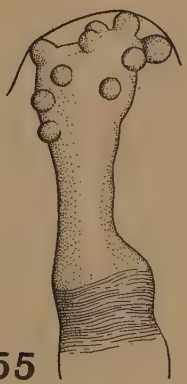
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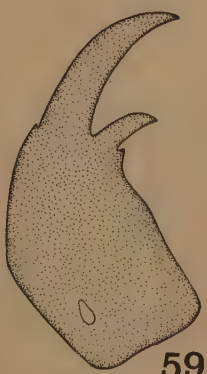
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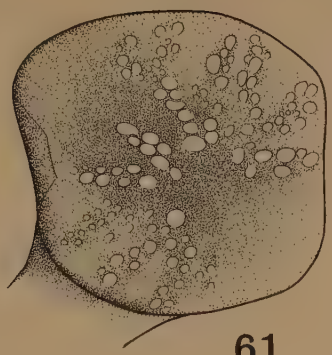

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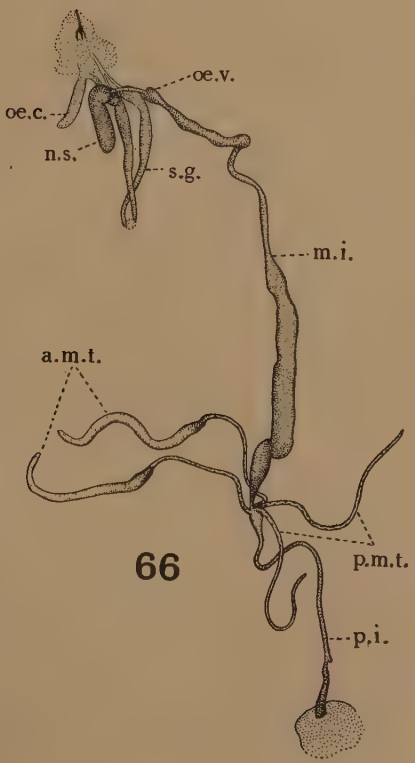

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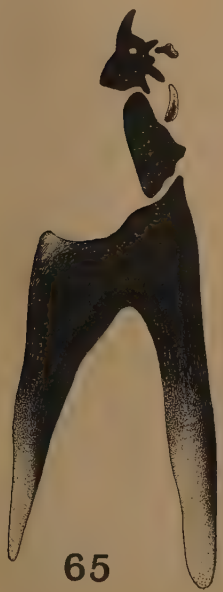

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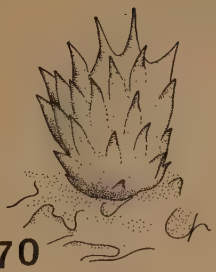

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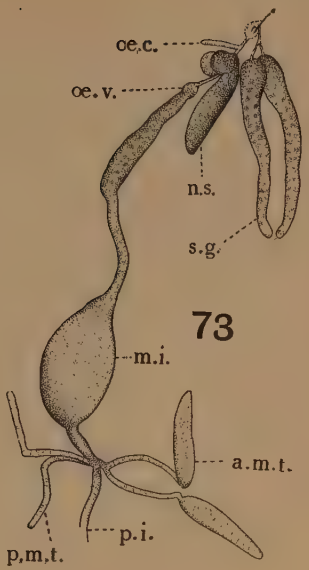

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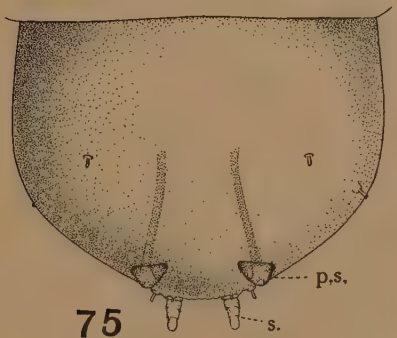

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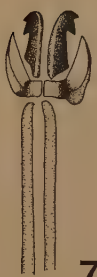

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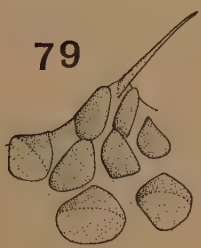

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ditions under which it was necessary to test the parasite[1]. An approximate idea of the number of aphids was however obtained by counting, on the one hand, the number of agglomerations or clumps, and, on the other, the number of individuals on what appeared to be an average-sized clump. The population was thus estimated to be approximately 112,000.

On June 8th, 100 aphids were removed, in batches of ten, from various parts of the tree. These were dissected and carefully examined; none contained parasites.

On June 9th, three twigs bearing aphids were removed from the *Aphelinus* insectary; all living aphids and aphid skins were removed, leaving on each twig 100 dead individuals containing parasites. These three twigs were attached to the three main branches of the experimental tree on June 13th. Numbered labels were now attached to 30 points on the tree in order to mark the points of collections and ensure a reasonable distribution of the samples taken. From each of these points 10 aphids were collected and dissected, but no eggs or larvae of *Aphelinus* were found. About 40 per cent. of the parasitised aphids placed on the tree had by that time issued from their hosts, and one adult was seen on a twig. On June 16th, practically all the *Aphelinus* had issued. One adult was seen, and in one of 200 aphids dissected one parasite larva was found. On June 20th and 23rd, 200 aphids were dissected; on June 27th, 30th, July 4th, 7th, 11th, 14th, 18th, 21st, 25th, 28th, August 1st, 4th, 8th, 11th, 15th, 18th, 100 aphids were also dissected. The percentage of aphids parasitised on the dates mentioned is shown in the accompanying graph. On August 18th, all of the sample of 100 aphids taken contained parasites. The dissections were then discontinued.

Toward the middle of June several torrential downpours of rain occurred. These washed away the greater part of the aphid "wool," and changed the appearance of the colony so much as to suggest that the population had been greatly reduced. A new estimate was therefore made, but the population was found to be about 135,000, so that it had, if anything, increased somewhat since the first count.

The curve of the percentage of parasitism (Fig. 1) is somewhat irregular. Its general trend is, however, fairly definite. It rises at first slowly, but more and more steeply toward its end. If larger numbers had been collected the irregularities would probably have been less pronounced, as they are almost certainly due to differences in local distribution, produced by chance.

Thus, as the graph shows, in 64 days from the colonisation of the tree by

[1] Every biologist who attempts to make quantitative studies of living organisms under natural conditions is constantly faced with this difficulty. If the conditions are really natural, accuracy is practically impossible; if accuracy is obtained, it is at the expense of the natural setting of the experiment. In attempting to measure the course of events, we determine a deflection, the amplitude of which may be practically impossible to measure. It is amusing to note that the physicists, having encountered something very like our ancient and familiar difficulty in their studies of the electron, have solemnly described it as the Principle of Indeterminacy, and have even attempted to extract from it a proof of the Freedom of the Will!

300 individuals of *Aphelinus mali*, a population of *Eriosoma lanigerum*, estimated to comprise about 112,000 individuals at the beginning of the experiment, was practically annihilated. The rapidity with which this result was obtained suggests that parasites must have come in from other areas. A few simple calculations will show, however, that this was not necessarily the case. If we assume that half of the individuals of *Aphelinus* were females (150), that each female produces two eggs per diem during a period of 20 days, and that

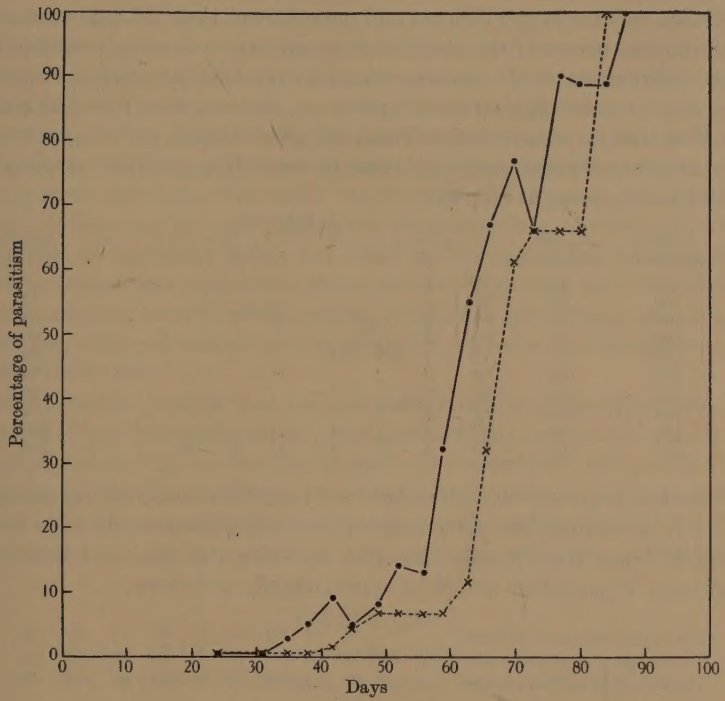

Fig. 1. Curves of the percentage of parasitism in (*a*) the case studied in this paper—solid line; (*b*) a theoretical case with an initial population of 90 females, a pre-reproductive period of 20 days, a reproductive period of 5 days, and an oviposition rate of two eggs per diem—dotted line.

the pre-reproductive period, from egg to egg, is 20 days, so that the adults from the eggs on the first day begin to oviposit on the 21st day, those from the eggs deposited on the 2nd day begin to oviposit on the 22nd day, and so on, the total population up to and including the 64th day would be about 3,400,000, which would be sufficient to exterminate a population over thirty times as large as the one treated.

Only 90 females, with their descendants, each producing two eggs a day during a period of 5 days, the pre-reproductive period being, as before, 20 days, will give rise, in 84 days, to a total population of something over 150,000

individuals. Since females of *A. mali* have been found to deposit from two to nine eggs each, living from 11 to 39 days, and producing a total of from 48 to 140 eggs during the reproductive period, the figures taken as a basis for the above calculations are well within the bounds of possibility.

In the accompanying chart (Fig. 1), the curve of parasitism corresponding to the theoretical figures in the last case mentioned has been plotted side by side with the real curve of parasitism, as given by the samples dissected. The two curves have the same general character, though the final rise of the theoretical curve is longer delayed and more abrupt than the true curve.

During the course of the dissections, an attempt was made to obtain data on the distribution of the parasite larvae in the host population. Until the percentage of parasitism attained 4 per cent., no hosts were found to contain more than a single parasite larva. From this point onward, one or more hosts in every sample dissected contained two or more larvae of the parasite, the detailed results being as follows:

Aphids dissected	Aphids parasitised	Aphids with 1	2	3	4	5	6	7	8	9	Total larvae
100	14	13	1	.	.	.	.	.	.	.	15
100	32	30	2	.	.	.	.	.	.	.	34
100	55	53	2	.	.	.	.	.	.	.	57
100	67	65	2	.	.	.	.	.	.	.	69
100	77	72	4	0	1	.	.	.	.	.	84
100	66	64	2	.	.	.	.	.	.	.	67
100	90	88	2	.	.	.	.	.	.	.	92
100	89	82	6	1	.	.	.	.	.	.	97
100	89	82	3	1	1	2	.	.	.	.	105
100	100	83	5	5	1	2	1	0	1	2	154

If the larvae present in each sample had been distributed among the hosts, absolutely at random, the percentage of parasitised hosts would have been a good deal lower than it was. Roughly speaking, the real and theoretical percentages of parasitism would be approximately as follows:

No. of parasites per 100 hosts ...	15	34	57	69	84	67	92	97	105	154
Percentage parasitised hosts ...	14	32	55	67	77	66	90	89	89	100
Theoretical parasitised hosts ...	14	29	44	50	57	49	59	62	64	78

It will be noted that the real percentage of parasitism is a good deal higher than the theoretical percentage of parasitism, which suggests that the parasites have some tendency to avoid ovipositing in parasitised hosts, like the females of *Trichogramma* recently studied by G. Salt (1934). It is, however, difficult to detect the remains of *Aphelinus* eggs or young larvae killed by older specimens, and further investigation on this point is required; especially since, in a sample of 100 specimens taken from the insectary, 52 individuals were found to be parasitised, of which 21 contained two or more larvae, so that the total number of parasites present could not have been less than 73—approximately the number required to give a 52 per cent. parasitism of the eggs were distributed at random.

The data presented suggest that, as observers in many parts of the world

have reported, *Aphelinus mali* is, under certain conditions, a very efficient parasite of the woolly aphis of the apple. The experiments carried on up to the present indicate, however, that under English conditions *Aphelinus* is not able to keep the population of *Eriosoma* permanently at a low level. In 1932 the writer placed a colony of the parasite on an isolated apple tree, heavily infested with the aphis, in his garden at Chalfont St Peter, Bucks., in early summer. Toward the end of the season it was practically impossible to find any live aphids on the tree; nevertheless, a heavy infestation developed early in the summer of 1933.

Furthermore, in the case discussed in this paper, the colony of *Eriosoma* appeared to have attained its maximum dimensions about the time the parasite was introduced; at all events, according to the rough estimate made, the population on June 28th was only 1·2 times its initial value on June 10th. This may, of course, have been due to mere lack of accommodation on the tree; but bare areas of bark were still visible. As Marchal (1929) points out, prolonged periods of heat and drought during the summer months are everywhere unfavourable to the woolly aphis, and check its increase either by their direct action or because they slow down the movement of the sap, and thus diminish the reproductive activity of the aphids, of which a good many abandon the upper branches and move down to the roots, where a more humid environment is obtainable.

It is therefore possible that the satisfactory results in the experiment here described were due partly to the fact that the increase of the aphids had practically ceased by the time the parasite was introduced, and partly to the existence of climatic conditions particularly favourable to the parasite. An earlier introduction might not have given such striking results. Further observations will, however, be made on the colony during the coming season.

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